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SCIENTIFIC REPORTS

FROM

THE GOVERNMENT INSTITUTE

FOR

INFECTIOUS DISEASES

THE TOKYO IMPERIAL UNIVERSITY.

Director: Prof. Mataro Nagayo.

For the Year

1922

Edited by

Dr. Yoneji Miyagawa.

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Preface.

The employment of the Japanese language having heretofore been one great obstacle in the way of the foreigners having an access to the result of the medical researches and investigations done by our Institute for Infectious diseases, we have decided to publish all research works in foreign language, which have been already communiated mostly in the monthly journal "the Japanese Journal of Experimental Medicine". It contains in its pages practically all the researches accomplished by the members of the Institute. The importance of this magazine is significant in the two outstanding features, first in that it reports the transactions instead of merely the abstracts of studies as the publication from the Medical Department of the National Research Council has been doing since 1922, remedying in that way one great defect which the latter institution has not as yet been able to rectify, and then secondly in that students are enabled to find in this single magazine grouped together all the different researches accomplished in our Institute which are directly or indirectly interrelated with one another, instead of their having to look up for them in different kinds of magazines in which each subject will have to be printed in the absence of this one. Moreover, although our institute, so far as its name is concerned, has to do with infectious diseases only, it is really the medical research nistitute in which medical nivestigatins in all its lines are being made. Especially. Having moreover lately added a new organization to meet the growing requirements of both the experimental works and the practical applications, we expect our magazine to become more copious in useful informations to the medical scholars as well as to the men engaged in practice. It is my pleasure to add that such a speedy execution of the decision was mainly due to the enthusiastic efforts of Dr. Y. Miyagawa, the chief editor.

Mataro Nagayo

The President of the Institute for Infectious diseases.

Dec. 28 th 1922.

CONTENTS.

BACTERIOLOGY AND SEROLOGY

Foreword to the reports from the Research Committee of the Institute concerning the investigation of influenza for past four years. M. Nagayo. 1

On the application of mixed vaccine as prevention against influenza. M. Nagayo. 5

On the immunity of influenza. M. Nagayo. 9

Dosis and antibody producing power in human body of the mixed vaccine of pneumococci and Pfeiffer's bacillus. K. Ishiwara, Y. Hiki, K. Nobechi and K. Kakinuma. 15

Experimental study with the blood of influenza patients. K. Nobechi, S. Nakamura, Z. Inouye, H. Nakajima, M. Nishibe, I. Nakada and S. Tenjin. 21

The bacteriological and serological study on influenza patients during the epidemic from January to March 1922. H. Nakajima, Z. Inouye, S. Nakamura and K. Nobechi. 25

An experimental study of filtering property of influenza virus upon certain animals. T. Abe, T. Ishikawa, M. Nishibe, I. Nakata and S. Tenjin. .. 29

An experiment study of inoculation with B. influenza on monkeys. T. Kunizaki, H. Nakajima, Z. Inouye and M. Nishibe. 35

The bacteriological examination of sputa in searching for Pfeiffer's B. influenzae and other as a possible factor in the etiology of epidemic influenza. T. Kunizaki, Z. Inouye and H. Nakajima. 41

The Pfeiffer's bacillus in sputum of tuberculous patients and its immunological investigation. S. Nakamura, J. Inouye, H. Nakajima and T. Kunizaki. 45

On the indol formation of B. influenza. N. Suzuki. 51

On the indol production of B. influenza. T. Kunizaki. 53

A new method of classification of Pfeiffer's bacillus by fermentation of carbohydrates. J. Nomura. 57

On the place of production of agglutinin for Pfeiffer's bacillus and its distribution and local immunity. J. Nomura. 61

An Experiment of the route of elimination of the bacilli by experimental paratyphoid infection nf rabbits. M. Arai. 65

On the reproducing paratyphoid B. infection by oral introducing in rabbit and its immunity. M. Arai. 69

Expedition for prevention of dysentry prevailed in a small island Toshima. T. Yamada, Z. Inouye and R. Kakinuma. 73

On two strains of cholera vibrio showing no motillity. K. Nobechi. 75

On the cholera strains encountered in Japan in 1921. K. Nobechi and T. Ishikawa. 79
A simplified method of finding tubercle bacilli in sputum, (especially the staining and collecting method. M. Tsutsumi. 83
The theory of the Wassermann reaction with special refference to the analogous specific reaction between heterophile (Forssman) antibody and tissue lipoids. T. Taniguchi. 87
Contributions on the study of the Forssman antigen and antibody. Ts. Iijima. 97
Studien über das hämolytische komplement, insbesonders zur ersatzbarkeit seiner einzelnen komponente. Ch. Nagoya. 131
La plej simpla metodo por serodiagnozo de sifiliso. M. Murata. 157
La plej simpla metodo por serodiagnozo de sifiliso. M. Murata. 161
Beiträge zu serologischen Untersuchungen über das enteral und parenteral eingeführte, artfremde Eiweiss. Y. Hiki. 163
Further studies on biological activities of serum elements against organ toxins. H. Wago. 193
Result of the prophylaxis of Weil's disease (spirochätosis icterohaemorrhagica) experimented both in Toyama and Chiba prefectures. Y. Tohyama. ..197
Experimental studies on the process of formation of vaccinal immunity. T. Ohtawara. 203
Ekzamenmetodo pri la efikeco de kupoksvakcino per enhaŭta inokulado ĉe kuniklo neperfekte imunigita. H. Kasai. 247

PATHOLOGY

On the function of the lymphocyte. M. Nagayo. 253
The organ or tissue cells as an irritant or toxin and the procedure of the creation of organ toxin. Y. Miyagawa. 259
An experimental study of the cells of the mucous membrane of the digestive tract as toxin or irritant, and its relation to the formation of peptic ulcer. Y. Miyagawa, H. Murai and M. Terada. 273
The influence of the mucous membranous cells of the digestive tract parenterally injected on the organ or tissue. H. Murai. 301
A study on kindney cells as toxin or irritative substance and its relation to the promoting of nephritis. J. Kimura. 339
Experimentelle Untersuchung über die Lymphocyten. K. Yokomori. 363
A clinical and experimental study of lymphocytes. M. Okada. 365
Leukocytes and influenza. M. Okada. 383
Morbid anatomy of influenza during 1918–1920. K. Sato. 391
Experimentelle Studien über die Pathogenese der Amyloidose. K. Sato. 393
Über die Leberveränderung bei Adrenalinvergiftung. K. Yokomori. 395
On the pathology and pathologic anatomy of experimental acidosis. T. Satow. 397

Beiträge zur kenntnis der biologischen Wirkung des Chlornatriums auf das Blut und die hämatopoetischen organe. S. Takamura. 399
The influence of some components of old tuberculin and tubercle bacillus on the progress of experimental tuberculosis. K. Yoshizawa. 427

CHEMISTRY

Über die glykolytische Kraft des Blutes. Y. Kawashima. 441
Über die glykolytische Wirkung des Harns. Y. Kawashima. 443
Über die Wirkungen der Spaltungsprodukte des polierten Reises auf den Froscherzen, besonders mit der Berücksichtigung von Vitamin (B). M. Ito. 453

Bacteriology and Serology

Foreword to the reports from the Research Committee of the Institute concerning the investigation of influenza for past four years.

By

Prof. **Mataro Nagayo**, M. D.

Director of the Institute.

Influenza which begun in Spain in 1918 soon spread all over the world, Japan was not free from its attack. This great epidemic was at its worst in our country about the month of November and December of the same year. During the years 1918, 1919 and 1920, influenza was most malignant, and it did not much abate until about the spring of 1921. It has been the mission and the duty of this institute that it should most actively engage itself in the work of preventing the epidemic and treating the patients on one hand, and to devote itself to the investigation of the virus of the disease and to the thorough going research of all that pertain to influenza on the other hand.

Japan like other countries greatly suffered from the pandemic that prevailed during the years 1889–1891. But as the medicine in our country was in an infantile condition then, only a scanty epidemiological description was given, and no record of the exact findings of scientific research was left to us. Since then our medicine has made a rapid progress, and to-day, after the elapse of 30 years, we rejoice that we are able to publish with our co-workers in the world the result of the research work on influenza in our country.

The view of scholars in our country about the problem of the virus of influenza like those in other countries are various. Some insist that the virus of this disease is Pfeiffer's bacillus. Some say it is the filtrable virus. Others would have some other bacillus than Pfeiffer's one. Again there is not lacking of those who have no positive assertion about the true virus, but would not ascribe the true cause of influenza either to Pfeiffer's bacillus or to other bacillii that have been enumerated. Our research work along this line during past four years has made us to incline to the idea that Pfeiffer's bacillus is not the true cause of influenza pandemy. Nevertheless we do not think Pfeiffer's bacillus is a harmless bacteria

in pandemic influenza. We admit that it has a fairly important rôle in the disease. In this respect, it has more significance than streptococcus and is comparable to pneumococcus. In other words, Pfeiffer's bacillus working with other bacillus would make the pandemic malignant in character, producing many cases of pneumonia and augmenting the rate of mortality. But except this fact, we have not been able to get any postitive proof that Pfeiffer's bacillus is the true cause of the pandemic. Moreover in the epidemic of this time, we and other investigators have made many thorough going researches which were not tried by the scholars at that time who found the cause of influenza in Pfeiffer's bacillus, when it was discovered by him at the time of the pandemic in 1891. According to the result of such researches, it is found that Pfeiffer's bacillus has points more of contrast than agreement with the virus of this disease. Hence it has become more improbable and more inconceivable as the true virus.

The members of the Influenza Research Committee of this institute, each working independently in their own lines, without interference, and without prejudices, sought to find out the virus of the disease. But when the results of various investigations are collected and combined together, the aimed-for object was still afar. There was not a single research the result of which could prove that Pfeiffer's bacillus is the true cause of influenza. If there were a single investigation whose result could definitely show that Pfeiffer's bacillus would be the virus, we should not have hesitated in regarding it as the true or probable cause of influenza, even if the investigations of the same subject according to other methods do not support this view in the same degree, provided there is no research whose result would absolutely contradict it, perhaps we might have changed our view which we have obtained from preceeding researches.

In 1919 or in the second year of pandemic we prepared a mixed vaccine of Pfeiffer's bacillus and pneumococci, and offered it to the world as the vaccinating material for preventive measurement. For we recognized that these bacilli had great share in aggravating and making malignant this disease, although they may not be the true cause of influenza, and that the use of this vaccine would make the epidemic lighter and decrease the death rate. This vaccine was applied to a large number of people. The result showed in the table had at least some preventive significance reducing the mortality.

The study of the filtrable virus has been one of the most important method of investigations during these days. In this respect, we have not been able to obtain any positive result as a few investigators in this land and in abroad would insist to have. But in this kind of investigation, we have to say that although our own research may end in negative result, we can not hence negate the result of general investigations. If even a few experiments which were carefully made and whose results were exactly estimated, could show the positive result, we must pay great attention to such investigation. But up to the present moment, we have not the sufficient reason to admit the views of the scholars of the world

who advocate the filtrable virus theory. More of the investigation along this line should be made hereafter.

The pandemic influenza is already gone. To our regret it is very difficult now to carry on the further investigation concerning the virus of this disease. The reason why we publish this report in which the result of our utmost endeavor for four years is recorded is primarily to make clear the scientific stand point of the Institute for Infections Diseases, by frankly describing the result of our investigations; and secondly, perhaps more emphatically, to give some light to those future investigators of pandemic again to come.

December 28. 1922.

On the application of mixed vaccine as a prevention against influenza.

By

Prof. **Mataro Nagayo.**

Director of The Institute and Head of the Pathological Depertmant.
(From the Influenza Research Committee of the Institute.)
(Published previouly in Japanese in the Journal of Hygiene and Infectious Diseases, October 1921.)

Since the prevalence of influenza two years ago, our institute has been making utmost efforts to find out the virus of the disease. But we have not been able to point out conclusively the definite virus yet. Neither the reports of many investigators concerning the virus could not give us the fundamental solution. It is our great regret that we have not get the preventive measurement which we can recommend to the world with an absolute conviction. As the virus is not yet ascertained, the inoculating materials prepared for the prevention of influenza are of various kinds. According to the views of different scholars, sometimes Pfeiffer's bacillus alone, sometimes its mixture with pneumococcus have been used. Again, the mixture of streptococcus, staphylococcus, and pneumococcus mucosus in different proportions has also been used. In order to meet the needs against the second prevalence of influenza since the latter part of last year, this institute prepared and sold out a mixed vaccine of Pfeffer's bacillus and pneumococcus. From the bacteriological and pathological findings of this disease which were obtained by the experience of last year's epidemic, we have known that these two kinds of bacilli are found in most of the influenza patients at present. Moreover in case of pneumonia, with regard to its symptom and progress, these bacilli have a significance on it that can not be overlooked. As we have recognized this fact with many scholars, and as there is no other more reliable preventive method, it would be a rational measure to prepare a vaccine from these bacilli and apply it to the present case. This is the reason why we have come to use the mixed vaccine.

Our mixed vaccine was prepared with the following method. Pfeiffer's bacillii of many origins and pneumococcus of four types and with all its kinds were separately implanted on the blood agar-plate and after being cultured for 24 hours in 73°C., they were collected and estimated, and suspended in the sterile physiological salt solution. For one hour, Pfeiffer's bacillii were heated at

55°C., and pneumococcii at 60°C. When their death was ascertained, they were diluted with sterile physiological salt solution which was mixed with 0.5% of carbonic acid, and the total bacterial amount in 1 c.c. is made 0.4 mg in which 0.2 mg each of Pfeiffer's bacillii and pneumococcii is contained. Applying vaccine to a large number of persons against influenza began with the recent epidemic. Accordingly, the effect of inoculation should be judged by the experience of this time.

We felt that it is our duty as a manufacturer that we should make investigation as to the effect of what we have prepared, and report the result. Thus in order to get the report of the effect of the injection of users as much as possible, I sent out investigation papers to the users of vaccine in hospitals, the municipal and village offices, police stations, schools and physicians, asking them to give the information about the number of the injected persons, their occupations, the place where they live, the kind and the dose of vaccine, the time of injection, the number of patients after inoculation, the condition of infection, the number of days between inoculation and infection. The summary of answers is shown in the table I.

Table I.

Injection of mixed Vaccine of Pfeiffer's bacilli and pneumococci, and the rate of infection.

		1 injection	2 injection	Total	
Total number of injected persons		6399	45310	51709.....A (−19228=32481.....B	
Non-infected persons		6296	44519	50815.....A (−19228=31587.....B	
infected persons	slight	75	506	584	65.3%
	moderate	12	172	184	20.6%
	severe	11	86	97	10.8%
	death	3	24	27	3.3%
	sum	103	791	894	100.0%
Rate of infection		A=1.6%	A=1.7%	A=894:51709=1.73% 894:5179=.... B=894:32481=2.75%	

Remark A=The total number reported, B=Total number after eliminatim of uncertain reports.

Slight Fever: two or three days.
Moderate Fever: about a week: generally not high.
Severe Fever: more than one week: generally high, clinically clear symptom of pneumonia.

The number of injected persons were 51709. Of them 894 or 1.73% were infected after one or two inoculations. We counted the infection after inoculation as such without regarding the time of the production of antibody, or the temporal

relation between inoculation and infection. The rate of infection after one inoculation was 103 : 6399 or 1.6%, and that of infections after two inoculations was 1.7%. But as the descriptions of some of the reports were too brief and imperfect, and as there should be obvious omission of some cases of infection after inoculation, I eliminated 19228 persons who fall under this category and took the remaining 32481 as the persons inoculated. Then the rate of infection after inoculation becomes 2.75%. Of 514 out of the total number, there were descriptions about the reaction after injectons. Of them, 174 were of slight local symptom, 44 of higher local symptom. Of 185 fevers, 128 were marked slight fever, 35 attained 38°C., and others described as high fever, or simply as fever, 47 had head ache, 37 general prostration, 12 no appettite, 5 were chilly, 3 had diarrhoea, 3 urticaria, 2 tinnitus aurium, 1 nausea, and 1 perspiration. The reaction of mixed vaccine both general and local is very slight compared to the old one.

In making investigations about the immunity of influenza. (I have made another report on it), I obtained some result about the relation of the injection of mixed vaccine and infection. It is shown in table II.

Table · II.

The comparison between the rate of infection of those are inoculated with mixed vaccine prepared by the Institute of Infectious Diseases and the rate of infection of these who are inoculated with the vaccine.

Number of injection	2 injection						1 injection						Injected		Non injected	
Prevalence	Second Prevalence												Second prevalence		Second prevalence	
Number of persons investigated	987						135						1122		3417	
Non injection	849						81						930		2022	
Number of days of infection after injection / Condition of infection	1–3	4–7	8–	Not clear	Sum	%	1–3	4–7	8–	Not clear	Sum	%	Total of infection after injection	%	Condition of infection	%
Slight	4	2	16	50	72	52.1	3	7	1	12	23	42.6	95	49.5	486	34.8
Moderate	0	3	14	19	36	26.1	9	2	3	4	18	33.3	54	28.1	430	27.9
Severe	2	2	15	8	27	19.6	4	5	0	2	11	20.4	38	19.8	410	29.4
Death	0	0	2	1	3	2.2	1	0	0	1	2	3.7	5	2.6	110	7.9
Total Number of infection					138						54		192		1395	

As this table is based on the report which from the nature of investigation laid more stress on the infected persons, it does not very well show the general

infection rate. But it gives some light as to the ratio of the relative severity of infection. Of 192 who were infected after inoculation, 95 (49.5%) were slight, 54 (28.1%) moderate, 38 (19.8%) severe, and 5 (2.6%) died. But of 1395 who were not inoculated and were infected, 486 (34.9%) were slight, 389 (27.9%) moderate, 410 (29.4%) severe, and 110 (7.9%) died. If the infected persons are divided into these four cases, and the percentage of each be taken, the note worthy fact that the rate of 'severe' and 'death' among those patients who were inoculated is very small will be disclosed.

But I do not mean that we can safely draw the conclusion about the rate of infection from the effects of vaccine injection thus far obtained. This is a very difficult problem. The figures appeared in the statistic are controlled by various complicated relations. Therefore we have to take many conditions into account. We have consider the age, constitution, occupation, the place of residing of inoculated persons, the time of injection i.e. whether inoculation was made before, or during, or in the latter part of prevalence.

We have to find out whether they have besides inoculation, been taking other preventives, such as mouth mask, gurgling of mouth, isolation of patients, carefulness in going out, or attending meetings etc.

Again we must know the ratio of the condition and the number of patients of the next epidemic all over the country and in the province where the injections were made. Moreover as the infection of preceeding year would leave a certain immunity, we must not neglect the investigation about this point. What I have to do here is simply to show the figures appeared in the reports. Many revisions which are necessary shall be made after more minute and exact investigations.

On the immunity of influenza.

By

Prof. **Mataro Nagayo.**

Director of the Institute and Head of the Pathological Depertment.
(From the Influenza Research Committee of the Institute.)
(Published previously in Japanese in the Journal of Hygiene and Infectious Diseases, October 1921.)

So the question. Do we get immunity after recovering from influenza? Various answer are given. Some would deny it altogether. Some admit immunity after the recovery from the disease, but they say its duration is not more than a few weeks. Others on the contrary would on the possibility of immunity of much longer duration and even say that the reason why relatively few patients in severe case were found among men over fifty in the epidemic of this time is to be found in the fact that majority of the people had suffered from this disease during 1889–1892, the time of its great prevalence. I have known majority of people who for certain had suffered twice or more than twice, from influenza in earlier (from 1918 to the summer of 1919) and later (from the latter part of 1919 to the present time) periods of this epidemic that began to prevail from two years ago. Again I have known majority of persons who had suffered only once in either of these two periods during which we have had two attacks from this epidemic. It is the matter of course that these should be differences according to the constitution and other conditions of individuals. But if the infection would give the man acquired immunity in any degree, the inquiry into it would have great significance in the estimation of the effect of vaccine injection in which we are now engaged. I felt myself great need of making an investigation about it. So I sent out investigation papers to acquaintances, physicians and others, asking them to make investigations as to the infection or non-infection of not only the individuals, but the whole member of the family if possible, in these earlier and later periods of prevalency.

By this means, I got the reports about 7800 persons by the time up to the latter part of March of this year (1920). If I divide them into family groups. I found on examination, that there were cases in which the whole member of the family were infected twice in earlier and later periods, but there were also cases in which some members suffered from the disease only once in either of the two periods, or only those who had suffered in the earlier period were free from the attack in the later period and all others were infected. Specially note-worthy

fact is the case of the families the whole member of which had vaccine injected to them in the time of second prevalence, but of these, the whole member of one family were infected and the whole member of another family were free from the attack; and afterward the fact was disclosed that those who were infected in the second prevalence, did not suffer in the time of first prevalence and those who escaped from the suffering in later period were affected with the disease in earlier period. Now it is difficult to settle whether the non-infection in such case is due to the acquired immunity after disease or to the effect of vaccine injection.

Investigation of the immunity of influenza.

Table I A.

On the basis of second prevalence.

Number of persons investigated								
			7444					
	Non-infection	2828		54.4%				
Non-infection	Slight	1155	(48.7%)					
	Moderate	784	(33.05%)	2373				
5200	Severe	433	(18.25%)	45.6%				
	Non-infection	584		48.4%				
Slight	Slight	261	(62.9%)					
	Moderate	110	(26.5%)	415				
999	Severe	44	(10.6%)	41.6%				
	Non-infection	505		7.00%		Non-infection	1777	69.9%
Moderate	Slight	114	(52.6%)			Slight	432(56.3%)	
	Moderate	70	(32.2%)	217	2544	Moderate	200(28.7%)	767
722	Severe	33	(15.2%)	30.0%		Severe	115(16.3%)	30.1%
	Non-infection	548		81.0%				
Severe	Slight	52	(42.3%)					
	Moderate	39	(31.7%)	123				
671	Sovere	32	(26.0%)	18.4%				
	Non-infection	140		92.1%				
Death	Slight	5	(41.7%)					
	Moderate	1	(8.3%)	12				
152	Severe	6	(50.0%)	7.9%				
Remark	Death rate:	152:7444=1.96%						
		152:2544=5.98%						

Slight — Fever for two or three days.

Moderate — Fever for about a week: not high fever generally.

Sever — Fever for more than a week: high fever generally, clinically clear symptom of pneumonia.

Investigation of the immunity of influenza.

Table I B.

On the basis of first prevalence.

Number of persons investigated								
			7873					
	Non-infection	2828		61.4%				
Non-infection	Slight	584	(32.9%)					
	Moderate	505	(28.4%)	1177				
4605	Severe	548	(30.8%)	38.6%				
	Death	140	(7.9%)					
	Non-infection	1155		72.8%				
Slight	Slight	261	(60.4%)					
	Moderate	114	(26.4%)	432				
1587	Severe	52	(12.04%)	27.2%				
	Death	5	(1.16%)					
	Non-infection	784		78.1%			Non-infection 2372	75.6%
Moderate	Slight	110	(50.0%)				Slight 415(54.1%)	
	Moderate	70	(31.8%)	220	3139		Moderate 217(28.3%)	767
1004	Severe	39	(17.74%)	21.9%			Severe 123(16.0%)	24.4%
	Death	1	(0.36%)				Death 12(1.6%)	
	Non-infection	433		79.0%				
Severe	Slight	44	(38.3%)					
	Moderate	33	(28.7%)	115				
548	Severe	32	(27.8%)	21.0%				
	Death	6	(5.2%)					
Death 129	129:7873=1.64%							
	129:3268=3.91%							

About these materials, I have made two fold investigations. In the first place, I made the infection of a person in the time of second prevalence (from the latter part of 1919 downward) the basis, and tracing upward to the time of first prevalence (from 1918 to the summer of 1919), examined infection or non-infection, the condition of infection of the same individual. In the second place, reversing the order I made infection, non-infection, the condition of infection of a person in the time of first prevalence the basis for starting and traced down to the condition of the same individual in the second prevalence.

The result is shown in table I A and B. Of 2544 persons who were infected in the time of second prevalence, 1777 (69,9%) had not been infected in the time of first prevalence, and 767 (30,1%) had been infected in the time of first prevalence also. Specially note-worthy fact is that the persons who

suffered from the severe case of infection in the time of second prevalence were most those who were not infected in the time of first prevalence. Of 152 who were died of the infection in the time of second prevalence, 140 or 92,1% were those who were free from the attack of the epidemic in the time of first prevalence. Again, when the condition of second prevalence is examined on the basis of infection or non-infection in first prevalence, it is found that, of 3139 who had been infected in first prevalence, 767 (24,4%) were reinfected in second prevalence, and 2372 (75,6%) were freed from the infection. The percentages of the severe cases and deaths of those who were infected in second prevalence were respectively 30,8%, and 7,9% in the case of non-infection in first prevalence, while they were respectively 16,0% and 1,6% in the case of infection in first prevalence also. From these facts, we can not but recognize immunity relation in them. I also made the investigation of the infection condition in two epidemic times, of 1365 applicants for Tokyo Higher Commercial School in March of this year and have obtained the following result shown in (Table II).

In this investigation, inquiry into the condition of infection has been made, But as it was made on the basis of oral answers of the applicants, I noted down simply infection or non infection in the table. The number of men investigated was not large. But surely their cases may be taken as a proof for the presence of certain sort of immunity. Of 1365 who were examined, 656 were not infected in any of those epidemic times, and all the rest had been infected once or twice in either one or both of these prevalences. Of 373 who had been infected in first prevalence, only 21 (5,6%) were reinfected in second prevalence, and other 352 (95,4%) were free from reinfections. Viewed on the basis of second prevalence, of 357 who were infected in second prevalence, 21 (5,9%) had been infected in first prevalence, and 336 (94,1%) had not been infected in first prevalence.

From these results, I conclude that although we must consider the individual susceptibility in the study of the infection of influenza, there is no room for doubting that the infection does give immunity to a certain extent. As to its duration, we can not hastily conclude. But from this investigation, we may easily infer that it may be about one year. Accordingly, in estimating the effect of various kinds of preventive fluid against influenza, that have been used in this epidemic time, it is very important to make investigations as to infection or non-infection of the preceeding year. For, although we can see a considerable decrease of patients after vaccine injections, we are not sure whether it may not be due to the immunity resulting from the infection in the preceeding prevalent time.

Table II.

Investigation of immunity of 1365 applicants for Tokyo Higher Commerecal School.

	On the basis of first prevalence			On the basis of second prevalence		
Non-infection	Total number 992			Total number 1008		
	Second time non-infection	656	66.1%	First time non-infection	652	95.0%
	Infection	336	33.9%	Infection	352	35.0%
Infection (including slight, moderare and severe cases)	Total number 373			Total number 357		
	Second non-infection	352	94.4%	First time non-infection	336	94.1%
	Infection	21	5.6%	Infection	21	5.9%

Dosis and Antibody producing Power in human Body of the mixed Vaccine of Pneumococci and Pfeiffer's Bacillus.

By

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The statistics on a large scale made by Prof. Nagayo (1) indicated that the effect of the mixed vaccine of pneumococci and B. Pfeifferi had not been satisfactory, and a suggestion was offered that we would get a greater effect by the increase of 50% of its contents.

The authors intended to determine the degree of the advantage, when this vaccine gained an increase of 50% of its dosis, measuring the antibody producing power in the human body.

Experimental.

A mixed vaccine was prepared in the following ratio of its components:

Components (Strains)		Ratio
Pneumococcus	Type I. "Hondo"	3.
,,	Type II. American II.	3.
,,	Type III. 899.	1,5
,,	Type III. "Tamura"	1,5
,,	Type IV. "Saizawa"	1.
,,	Type IV. 1039	1.
B. Pfeifferi	"Tamiya A"	1.
,,	"Tamiya B"	1.
,,	"Kiuno"	1.
,,	"Takaki"	1.
,,	7.	1.
,,	8.	1.
,,	9.	1.
,,	12.	1.

Two sorts of vaccines varying in 50% of their contents but in the same constitution, as mentioned above, were prepared: (a) vaccine contained 0.4 mg. in 1 cc. and (b) vaccine 0.6 mg. in 1 cc.

The volunteers were divided into two groups: A and B. The members of A group received injection of (a) vaccine, and that of B group (b) vaccine. Two inoculations were given subcutaneously; 1 cc. was given at first, and after one week 2 cc. was injected.

Blood was removed from each person immediately before the first inoculation and 10 days after the second inoculation. Among 33 persons inoculated, 7 persons could not receive the second injection; their second removing of blood was performed 10 days after the single inoculation. Two persons having obstacles, could not furnish the samples of blood.

The serological study performed on the sera, separated from the withdrawn blood, consisted in agglutination test and complement fixation test for pneumococci and B. Pfeifferi, and measuring of the protective power against pneumococci. The protective power against Pfeiffer's bacillus was not tested, because the virulence of B. Pfeifferi can not be made sufficiently high to be suitable for this test.

Agglutination test.

Agglutination test for pneumococci was carried out for each type of it with the living emulsions of the strains used in preparation of vaccine. Reading was made, after keeping in an incubater for 18 hours.

Agglutination of Pfeiffer's bacillus was tested with the mixed antigen of all strains selected for vaccine, following closely Odaira's method (2).

Sera obtained before inoculation were tested as control, and they showed the results as mentioned in table 1.

Table 1.

Agglutination of the sera obtained before vaccination:

Antigen.	Highest dilution showing agglutination.
Pneumoc. Type 1.	10 ....40.
,, Type 2.	10 (−) .....40 (+).
,, Type 3.	10 (−).
,, Type 4.	10 (−) .....20 (+).
Pfeiffer's bac.	10 (−) .....20 (+).

Reflecting upon the anamnesis of experienced influenza and received inoculation, no causality was recognized between the potency of normal antibody and the experienced influenza or inoculation of the same vaccine given one year before.

Agglutinin titers of the sera obtained ten days after injections are summarized in table 2.

Table 2.

Agglutination of the sera obtained from the inoculated.

Group	Antigen	Highest dilution showing agglutination. Once inoculated.	Twice inoculated.
A.	Pn. Type 1.	20.....160.	80.....320.
	„ Type 2.	20..... 80.	40.....320.
	„ Type 3.	10 (—).	10 (—)
	„ Type 4.	10.....160.	20.....160.
	Pfeiff. bac.	10..... 80.	20.....160.
B.	Pn. Type 1.	40..... 80.	80.....320.
	„ Type 2.	40.	20.....160.
	„ Type 3.	10 (—)	10 (—) On one case 10 (+)
	„ Type 4.	20..... 40.	80.....320.
	Pfeiff. bac.	10..... 20.	20.....320.

No difference of the effect can be seen between (a) vaccine and (b) vaccine, either after single inoculation or after two inoculations.

A wide divergence in the individual responses was markedly revealed.

And parallelism between the potency of normal antibody and the response to vaccination was not distinct.

Complement fixation test.

Complement fixation test was carried out with the mixed antigen of four types of pneumococci and with the mixed antigen of all strains of Pfeiffer's bacillus used in vaccine. Antigen was used in quantity of ½ of the under-inhibitory dosis. For the hemolytic system goat hemolysin and goat corpuscles were adopted. The carrying out of the test followed the ordinary method.

Most of the sera obtained before inoculation, reacted in dosis 0.1 cc. negatively against both of the antigens. One serum reacted positively in dosis 0.05 cc. against both antigen.

Reactions of the sera obtained after inoculation are as summarized in table 3.

Table 3.

Complement fixation of the sera after inoculation.

Group	Antigen.	Smallest amount of sera showing fixation. Once inoculated.	Twice inoculated.
A.	Pneumococci.	0,1 (—). .....0,05 (+)	0,1 (—) .....0,1 (+).
	Pfeiff. bac.	0,1 (—).....0,025(+).	0,1.....0,025
B.	Pneumococci.	0,1	0,1.....0,05
	Pfeiff. bac.	0,1	0,1.....0,026

Thus the complement fixation test also indicated to us that (a) vaccine and (b) vaccine have the same ability to produce antibody.

Test of protecting power.

Protective power of the sera was examined with Type I, Type II and Type III of pneumococci. Using 18 hours culture on the slant beef blood agar, the smallest amount of virus, which could kill mice weighing ca. 12 gr. in 24 hours, was determined as D. L. M., after the virulence had been made sufficiently high to use the virus for this test. D. L. M. of Type I, Type II and Type III were respectively 1/500 mg., 1/3000 mg. and 1/500 mg., when they were used in test.

Virus in quantity of 2, 5, 10 and 20 times of D. L. M. were mixed with 0.1 cc. of sera and introduced intraperitoneally into mice, and observed during 5 days. Three mice were used for each dosis of virus.

Almost no protective powers were revealed by the sera obtained before inoculation in dosis of 0.1 cc. 0.1 cc. of the sera obtained after second injection could protect mice against 5–20 times of D. L. M. of Type I, 2–10 times of D. L. M. of Type II and sometimes 2–5 times of D. L. M. of Type III. Some sera failed to reveal protective power.

And in this experiment also, no difference of effect was discernible between (a) vaccine and (b) vaccine.

Cecil and Austin (3), Whitmore (4) reported on the sera obtained from those treated with pneumococcus vaccine, and the results obtained by them are more effective than ours. But they used more virulent strains than we, and the more virulent the test virus is, the more marked results will be. Using not so high virulent strains, Howell (5) could not reach effective results. And the method used by them was different from ours.

Summary and discussion.

Sera of 31 person inoculated with two sorts of vaccines varying in 50% of its contents, were serologically studied. And the two vaccines were proven to have no difference in their antibody producing power. Cecil and Austin (3) reported the same fact on pneumococcus vaccine. But these facts indicate merely the datum on the antibody production in the serum, and not that, on the resistance.

One of the authors once treated guinea-pigs with two sorts of cholera vaccine varying in 50% of their dosing; their doses pro kilo for guinea-pigs were made to correspond to two times of the dosis pro kilo generally used for man. After completing two of the subcutaneous inoculations, the resistance of the guinea-pigs against intrapertioneal injection of virulent cholera virus in various doses was tested. And no difference of resistance was recognized between two groups treated with the two different vaccines as mentioned above.

The fluctuation in 50% of vaccine dosing will not show any difference on its whole effect, that is not only on its antibody producing power, but also on its resistance elevating power.

But in the matter of the duration of immunity, what rôle does the fluctuation of vaccine content play? That is a question.

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Experimental Study with the Blood of Influenza Patients.

By

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In the systematic study on influenza in our institute, authors had been charged to make research on the problem as given in the title. The aetiology of influenza stands still in chaos. Studying the epidemiology and the clinical features, we approve the opinion that the infecting agent of influenza must be similar to that of measles. The virus of the measles exists in the respiratory organs and in circulating blood. Investigations dealing with the microorganismal excitants in secretions of the respiratory organs of the influenza patients have been extraordinarily numerous, but no unanimous conclusion has been made. And the studies to seek the influenza virus in the influenza patients blood are rather rare. In the field of the last mentioned studies, Yamanouchi and his co-workers (1) Dujarric de la Rivière (2) declared they had obtained positive results, but McCoy (3) and Rosenau (4) gained negative results. The objects of their experiments are all human bodies, but human bodies are not suitable for this experiment, because seemingly never attacked men, who are selected for experiment may have acquired immunity by slight unnoticeable influenza. Experiments upon animals were tried by Nicolle et Lebailly (5), Gibson and his associates (6), but their experiments with the blood are of too small a scale to deduct a definitive conclusion.

We attempted to determine the existence of the influenza virus in circulating blood by animal experiments and to get some light upon the obscurity of the influenza aetiology.

Experiment.

Materials employed in these experiments were obtained from the cases of the epidemic influenza in Tokyo in February 1922. The epidemic was mild and had little tendency to pneumonic complication, but had typical features of influenza.

Each samples of blood was divided into two parts. One part of it was

citrated, the other defibrinated and each experimental animal received both parts of the blood. Only the patients in early stages were selected as blood furnishers, for influenza is said to be infective only in the earlier stage.

As experimental animals, rabbits, guinea-pigs, monkeys and new-born, still-sucking dogs were used.

The object of the experiments was, in the first place, the determination of the possibility of transmission of influenza virus from man to animal. Transmission experiments were seemingly unsatisfactory but the virus might have not enough virulence to produce any notable symptoms in the first generation, and it might gain adaptation and increase of pathogenic activity in further generations. Therefore passage experiments from animal to animal were tried in the second place. Finally the plan included a study as to whether the patients blood could have any effect on the pathogenic activity of the pneumoccoci and Pfeiffer's bacillus, which were considered by us as secondary invaders.

In order to draw the result, daily measuring of rectal temperatures, daily counting of the leukocytes and observations of general symptoms were made. Animals sacrified and those dying subsequent to inoculation were autopsied, and gross and microscopic examination of the organs were carried out. In order to determine the existence of any intercurrent infection, and of injected pneumococci and Pfeiffer's bacillus, cultures were made from heart blood and internal organs.

Experiment. 1. Transmission experiment.

Patient's blood was collected seven times and we were able to perform seven series of experiments. One of them was done, fortunately, with the blood of a single patient in the incubation period.

Among six monkeys used in this experiment, 10 cc. and 25 cc. of the blood were introduced intravenously into two, respectively; 5.5 cc. and 10 cc. intratracheally into two; 10 cc. intraperitoneally into one; and 2 cc. intraspinally into one. Clinically one monkey showed leucopenia, and another one fever, but besides these no remarkable change was recognized.

Into 3 dogs, 3–4 cc. of blood was injected intravenously, and subsequently two showed leucocytosis but no fever. At the autopsy of one, perivascular infiltration of mononuclear cells in lungs was noticed.

Further 12 rabbits were used for experiments; material was given intravenously or intratracheally in quantities varying from 1 cc. to 10 cc. No noteworthy clinical symptoms were encountered. At the autopsy of six, we noticed on four peribronchial infiltration of mononuclear cells, on four perivascular infiltration of the same cells in liver and that in the Glisson's capsules, and on three hyperemia of trachea; one of the last three had been injected intratracheally but the other two intravenously.

Among eight guinea-pigs, to four 0.5 cc.–2 cc. of material were given intravenously, to three 0.25–0.5 cc. intratracheally and to one 0.5 cc. intraperioneally. No unanimous signs could be seen clinically. At autopsy of six, we noticed on three,

peribronchial and perivascular infiltration of mononuclear cells in the lungs, on one, that in the Glisson's capsules, on three, haemorrhages in the lungs, on four, pneumonia : two of the four, which showed pneumonia, complicated with bronchitis purulenta.

Experiment. 2. Passage experiment.

From the animals used for experiment 1, blood was removed by heart puncture and treated as in the case of the patients blood and transferred to new animals. In the same way, further passages were tried. Through careful cultural controls of the with-drawn blood any intercurrent contaminations were excluded.

With three monkeys, one dog, 11 rabbits and 16 guinea-pigs, passage experiments from the second to the sixth generations were carried out with the virus of various series. And the result was entirely similar with that of experiment 1. No signs of increased pathogenic activity were discernible.

Experiment. 3. Coinjection experiment of blood with Pneumococci and B. Pfeifferi.

Two monkeys, 1 dog, ten rabbits and nine guinea-pigs were treated with the patients blood or the blood of the passage animals, and concurrent with the blood introduction or after varying intervals pneumococci and the Pfeiffer's bacillus were inoculated to them intratracheally. The strains of the pneumococci and B. Pfeifferi used in this experiment were those freshly isolated from the same epidemic. Both or one of the two species of the microorganisms were injected into one animal in varying doses from 3 loopfulls to 4 plates.

The animals revealed, subsequently, fever of various types, varying changes in the number of leucocytes and ended frequently in exitus letalis. Histopathologically they showed marked pnumonia in various forms.

Experiment. 4. Control experiment.

(a) Normal animals.

The temperature of the guinea-pig is unstable concomitant with the change of the outward temperature. Therefore we took daily the temperature of some normal animals as control. The leucocytes counts of guinea pigs gave always relatively constant results and we could adopt the leukocytic change as a pathological datum. At autopsy, among four rabbits, one revealed tracheal hyperemia, one peribronchial infiltration of mononuclear cells, one perivascular infiltration of the same cells in the liver and the same in the Glisson's capsules, and the fourth revealed no change. Among ten guinea-pigs sacrified, two showed peribronchial and perivascular infiltration of mononuclear cells in the lungs, and four, which were killed by occipital blow, had haemorrhages in the lungs but the others killed by shot or air embolus had no haemorrhage. Atelectasis were often encountered as other autors have pointed out, especially on guinea-pigs.

(b) Experiment with the blood of healthy persons.

Blood was obtained from four healthy persons and injected intravenously into four rabbits and five guinea-pigs. At autopsy, among four rabibts, on one,

tracheal congestion could be recognized, on another peribronchial and perivascular infiltration of mononuclear cells in the lungs, on another the same in the Glisson's capsules. Among four guinea-pigs autopsied, peribronchial and perivascular infiltrations of mononuclear cells in the lungs were discernible on one. Pneumonia, however, developed in none of them.

(c) Experiment simply with Pneumococci and B. Pfeifferi.

Three rabbits and four guinea-pigs were used for this test. No difference in clinical and pathological findings could be seen between this experiment and experiment 3.

Summary.

The whole experiment using 9 monkeys, 5 dogs, 38 rabbits, and 47 guinea pigs can be summarized as follows:

1) All animals treated with the blood of influenza patients, showed no specific unique symptoms. A monkey into which 25 cc. of blood from a patient, who showed typical symptoms, had been given, had even no sign of influenza. One sample of blood from a person in the incubation period resulted also in no specific change.

2) It seemed to be noteworthy that perivascular infiltration of mononuclear cells as a pathological finding, was recognized in relatively higher frequency, for the lesions of the general cardiovascular system is a pathognomonic sign of influenza. But the same finding was able to see in comparatively lower frequency on control animals treated with normal blood and even on normal animals. Pneumonia was encountered only on guinea-pigs treated with the patients blood, but their types were not unanimous.

3) The influenza virus supposed to exist in patients blood did not seem to increase its virulence by animal passage.

4) The animals treated with blood together with pneumococci and Pfeiffer's bacillus showed pneumonia of various types, but they had nothing specific, compared with the changes on animals treated simply with the mentioned microorganisms.

Conslusion.

The existence of specific influenza virus, which may also cause any specific change in experimental animals was not able to be detected in patients blood during the epidemic in Tokyo in February 1922.

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The bacteriological and serological Study on Influenza Patients during the Epidemic from January to March 1922.

By

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Sputa and throat swabs of influenza patients collected principally from soldiers during the epidemic from January to March of 1922 were subjected to the bacteriological examination.

Out of 109 Specimens comprising 96 sputa and 13 throat swabs, Pfeiffer's B. was found in 53%, pneumococcus in 34%, staphylococcus in 70%, streptococcus haemolyticus in 22,9% and non-haemolytic streptcoccus in 22,9% of the specimen. Methods for the examination were the same as those employed by Kunisaki, Inouye and Nakajima on the similar investigation, mentioned in their report.

As to whether higher percentage of the propagation of the Pfeiffer's bacilli can be gotten from the cases of the later stage of the disease, it was not decidely demonstrated in our observation; out of 69 specimens obtained in the first week of the disease, the bacillus was positive in 33 cases (47,8%), while it was found in 26 cases (65,2%) and in 14 cases (57%) in those examined in the second week or after 15 days of the disease respectively.

On repeated examination of materials from the same patients, the constant relationship between the propagation of the bacilli and stages of the disease was not shown.

On the other hand, 24 strains of Pfeiffer's bacilli, isolated during this examination were examined for their agglutinability to 5 antisera from rabbits, which had been immunized with different strains of the bacilli obtained in the previous epidemic (1918–1920) only 3 specimens were agglutinated by such sera with relatively high dilution (640 times or 320 times), but the other 21 strains gave practically negative results except a few instances, which were agglutinated with 80 times of the dilution of the antisera. Moreover, a certain strain of the bacilli was agglutinated with the dilution of 640 times of one antiserum, but negative or slightly positive with the other antisera.

Therefore, Pfeiffer's bacilli, which had been isolated from the different sources

could not be considered as homologous from the immunological point of view.

It is known that sera of influenza patients give rise to positive agglutination, from time to time, with corresponding microorganisms. Hence, 14 sera from the different patients, of which some were complicated with broncho-pneumonia, were tested for the content of the agglutinines for the varied strains of influenza bacilli; but the majority of the sera exhibited only trace of the content of the specific agglutinines except a single specimen, which agglutinated some strains of the bacilli till 640 times of the dilution. Neither parallel increase of the agglutinine content along with the duration of the disease, nor any appreciable specificity of agglutinine to homologous strain which is isolated from the same individum, could be observed.

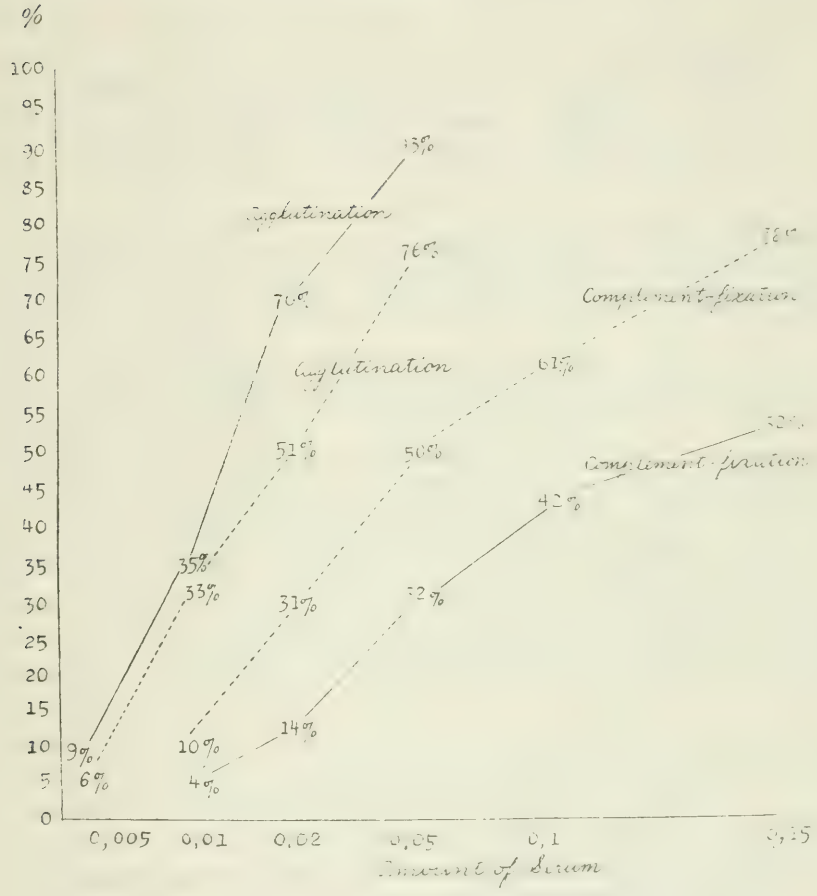
The same experiment on 6 sera from non-influenza patients (comprising 3 suffered from pleurisy, 2 from anchylostomiasis, and one from rheumatism) has shown that one serum gave difinitely positive result up to the 160 times and 2 sera to 80 times of the dilution.

A positive complement-fixation test has also been observed between some sera of influenza patients with a certain strains of Pfeiffer's bacilli (19 cases were examined), but here again we could not acknowledge any support to consider that Pfeiffer's bacilli and the sera of influenza patients stand on some direct specific relationship. That is, as in the case of the agglutination test, here exsits no constant relationship between the content of the complement-fixing body and the stages of the disease; furthermore it was not always observed that the sera of influenza patients revealed more marked complement-fixing reaction with the corresponding strains than with heterologous, furnished by other influenza patients or those suffering from pulmonary tuberculosis. Even in the sera of patients from which germs inquestion had been isolated abundantly, the antibody content was quite irregular.

Examination of 9 different sera from non-influenza patients demonstrated that complement-fixing antibody has also been contained in such sera nearly the same in frequency and in quantity, as it is shown graphically in the table.

Hence it would be concluded that Pfeiffer's B. can not be accepted as a possible factor in the etiology of epidemic influenza from the bacteriological and immunological point of view.

Table showing graphically the percentage and intensity of agglutination and complement fixation of Pfeiffer's bacilli with sera from influenza (————) or non-influenza patients. (.....)



An experimental Study of filtering Property of Influenza virus upon certain Animals.

By

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The etiology of influenza is not still definitely resolved, whereas the investigation of filtrable virus as its cause is an inevitable matter in this point, and up to the present time a considerable number of research works are already communicated from this view point.

Kruse described a filtrable virus as the cause of common cold, which is assertained by Foster and Dold. Kruse and Selter inoculated with the filtrate, made from influenza sputum in man with positive result.

According to the experimental results of Angerer, Leshke, Fejis Bradford and his co-workers, Gibson and his co-workers, Olitsky and Gates, Loewe and Zeman, the filtrable body is cultivated and thus cultivated substance is inoculated in man and in certain animals with positive results. Nicolle and Leboilly, Hall, Yamanouchi and his associates got nearly the same result. On the contrary to the above mentioned positive results, Schmidt, Maitland and Braham, Matsunami and his coworkers, and Ishiwara studied the same promblem, and concluded that the influenza virus si not filtrable.

We studied also on the filtrable virus of influenza during 1920–1922 with certain materials from the influenza patients in our Institute Hospital, the Yokosuka Naval Hospital, the First Military Hospital, the Medical Office of Imperial Guard and the Izumibashi Charity Hospital. The mixture of sputum and gurgled water with physiological saline solution was filtered at first with absorbent cotton wool or ordinary filter paper and thus made filtrate passed through Berkefeld N by negative pressure with water current pump of mercurial negative pressure 80 m.m. in column, by which procedure about forty droplets came out from the filter in every minute. Thus treated Berkefeld filtrate was inoculated intravenously or intratracheally into guinea-pigs, rabbits, in a majority of cases and a few monkeys.

The experiment upon guinea-pigs.

Guinea-pigs were injected intravenously or intratracheally with 1 c.c. of filtrates and were observed clinically 2–7 days long and then were killed by a

blow on the neck, shooting by air-gun or air-emboli, from the clinical point of view, they showed for the most part no symptoms, except a slight leukopenia in a few cases.

Pathological anatomical findings.

The majority of cases showed some pathological changes, which were without differences in intravenous and intratracheal injection.

The particular change was seen on lungs, showing edema, hyperemia, and bleeding in various degree, which appeared in some cases nearly on whole organs, but in some others very slight having pin head hemorrhagic patches in few points. In the cortical substance of adrenals there are often hemorrhages in the cases inoculated intratracheally; pleura, liver, spleen and kidneys showed no noticeable change.

Such hemorrhage in adrenals was never seen on rabbits and monkeys inoculated intravenously and intratracheally and also in control animals.

Microscopical appearances of organs.

The principal change of lungs consisted of hyperemia and hemorrhage, with dilatation of the intervesicular capillary and being full of erythrocytes in intervesicular tissues and in airvesicles. Perivascular and peribronchial infiltration with mononuclear cells for the most part and a few polynuclears was seen and megacaryocytes were observed often in blood capillaries. In small bronchus there were occured in some places desquamation of epithelial cells and in the centre an extravasation of erythrocytes, and in exceedingly implicated cases epithelial desquamation was seen in larger bronchus and also in trachea.

In most cases there was on the tracheal mucous membrane more or less congestion and round cell infiltration in the submucous layer.

Such tracheal change was especially distinguished in the intratracheal inoculated cases, and it was perhaps attributable to the method of inoculation. In the cortical substance of adrenals small hemorrhagic patches were seen with necrotic foci on their surrounding parenchym and often here perivascular infiltration with mononuclear cells. In other organs there was no noteworthy change.

Virus passage through guinea-pigs.

In order to make virus passage through guinea-pigs, we made the inoculating material in emulsion from a lower lobe of a lung which was severely affected. Such emulsion made in 30 c.c. of physiological saline salution with a lobe of a lung was filtrated by Berkefeld N. We inoculated the filtrate into guinea-pigs in two series, namely in first way intravenously and in second intratracheally.

We employed just the same method in order to further inoculation. Experi-

mental animals were killed always after elapse of 3–5 days from the inoculation for examination of the result, the intravenously inoculating series showed the positive results until seventh generation.

We were able to see positive results in 13 cases showing in lung the above described particular change among 20 experimental animals, dubious in 4 cases and negative in 3 cases. The intratracheal inoculating series continued the eighth generation, showing positive results in lungs in 11 cases among 17 experimental animals, dubious in 4 cases and negative in 2 cases. We made as a control investigation a following experiment, namely autopsycal examination of 11 normal guinea-pigs killed by a blow on the neck, of 7 killed with chloroform and of 5 killed by shooting with an air-gun. Among those animals we found only slight hemorrhage in a few points of lungs of 4 animals killed by a blow on the neck and of a guinea-pig killed by chloroform, but in all animals no trace of hyperemia, and no hemorrhage in adrenals. We made the second control study that the filtrate from patient material was heated at 60°c and 30 minutes long and then was inoculated intravenously with its certain amount into 9 guinea-pigs, 5 among which showed slight hemorrhage in lungs, but no change in adrenals. In the third control the Berkefeld N filtrate with gurgled fluid from healthy person in physiological salt solution was inoculated intratracheally into 4 guinea-pigs being shown in all no pathological appearance in lungs and in other organ. As the fourth control we took such an experiment, in which the filtrate made from normal guinea-pig's lung or physiological saline solution was inoculated intratracheally into guinea-pigs, which had no change at autopsy.

Experiment upon rabbits.

The rabbits were inoculated intratracheally or intravenously with 5 c.c. of filtrates made from patient's material and were killed by a blow on the neck or shooting with airgun after elapse of 2–7 days.

With regard to the clinical appearance, we could not find no noteworthy symptoms in any cases, no leukopenia, no conjunctivitis, no rhinitis and no diarrhea as described by Olitsky, but slight increase of body temperature in a few cases of animals inoculated intravenously with filtrates.

Morbid anatomy.

There are always principal findings in lungs, namely hyperemia on the mucous membrane of the trachea, especially of the animal inoculated intratracheally, and edema, hyperemia, and hemorrhage in lungs, for the most part in the lower lobes always severely affected, which were in main points the identical infings to those of the guinea-pigs. In other organs there was no notable change. In thus treated animals we could find a distinguished change in lungs in 6 out of 10

animals inoculated intravenously and in 7 out of 12 inoculated intratracheally, and remarkable hyperemia on the mucous membrane of trachea in all of the last cases.

Microscopical appearance.

In lungs, slight edema, hyperemia, and hemorrhage were seen in every site of change observed by naked eye. Beside these, slight infiltration of mononuclear cells and polynuclear ones in few amount was occurred in peribronchial and perivascular tissue.

In trachea wall also, hyperemia and perivascular infiltration of mononuclear cells were seen. No particular change in adrenals, liver, spleen and kidneys.

Virus passage through rabbits.

We were able to transmit the filtable virus till 7th generation in the experiment of virus passage by 12 rabbits used. In each generation we observed a partial change described above in lungs and in last generation negative result in 3 cases and dubious in 2 cases. We did by the time also certain necessary control experiment mentioned above in guinea-pig's study, in order to confirm our ordinary experimental result.

Experiment upon monkeys.

In order to confirm the above described findings, we used as the last experimental animal two kinds of monkeys, namely maccacus fuscutus and rhesus, without any notable pathological change at autopsy. It is interesting to describe briefly the whole experimental course upon monkeys.

1) Maccacus rhesus No. 7 was inoculated intratracheally with 5 c.c. of the Berkefeld N filtrates made from patients materials.

No increase of bodily temperature and no pathological change to be seen by the autopsy of animals killed by pistol shooting on fifth days after inoculation. On the contrary, slight leukopenia was observed, namely the leukocytes estimating shown 11200-10602 on previous day of inoculation and on second day after treating 5500 and on third day 4700.

2) Maccacus fuscatus No. 10 was inoculated with 5 c.c. of the Berkefeld N. filtrate made from the lung of the monkey No. 7.

The monkey showed also slight leukopenia, namely, the leukocytes counting as follows:

19100 ..... 20900 on previous day
23550 ..... on the 1st day
15400 ..... on the 2nd day
16250 ..... on the 3rd day

14200 ..... on the 4th day
20350 ..... on the 5th day
19100 ..... on the 6th day

3) Maccacus fuscatus No. 11 was inoculated intratracheally with 5 c.c. of filtrates made from the lung of the monkey No. 7 mixed with half of a slant culture of Pfeiffer's bacillus and Pneumococcus mucosus.

The estimating of leukocytes showed also slight leukopenia as following number.

22700 ..... on the previous day
24700 ..... on the 1st day
14900 ..... on the 2nd day
11400 ..... on the 3rd day
9000 ..... on the 4th day
11300 ..... on the 5th day
11300 ..... on the 6th day
12800 ..... on the 7th day
18300 ..... on the 8th day

The monkey was killed on 8th day by pistol shooting. In the lungs and in other organs there was no noticeable change, and Pfeiffer's bacillus and pneumococcus mucosus were not cultivated from lungs. On the contrary to this, the monkey had a small pustuleous nodule on the neck occured by pneumococcus mucosus and not by Pfeiffer's bacillus.

4) Maccacus rhesus No. 8 was inoculated intravenously with 5 c.c. of the filtrate made from patient's materials. Clinically no change, this is to say, no increase of fever and no leukopenia.

On the 12th day after the first inoculation it is injected intratracheally again with 5 c.c. of filtrates from Patient's material mixed half of a slant culture of Pfeiffer's bacilli, showing still no note-worthy clinical phenomenon and also pathological change.

5) Maccacus fuscatus No. 3 was injected intravenously with 5 c.c. of the filtrate made from the patient's material and on 8th day again injected with 5 c.c. of the filtrate mixed with half of a slant culture of Pfeiffer's bacillus, showing no clinical symptom and no pathological finding.

6) Maccacus fuscatus No. 4 was treated by the same manner as that of the monkey No. 3, showing completely negative result.

An Experimental Study of Inoculation with B. influenzae on Monkeys.

By

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and
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Though we know a great number of articles with regard to the etiologic problem of the pandemic influenza in 1918–1920, we are inclined at the present time to the opinion that on causative agent has been so far brought in light with certainty. The dispute is concerned more particularly with the etiologic rôle played by the B. influenzae in the pandemic. Among many investigators who ascribe its etiologic rôle to B. influenzae, it is, so far as we know, Blake and Cecil, that have made seemingly the most striking proof in this respect with their experiments on monkeys.

Twelve monkeys were inoculated with B. influenzae on the mucous membrane of the upper respiratory tract and contracted without exception a disease resembling to influenza in man; moreover broncho-pneumonia was observed in three cases. In seven among ten monkeys inoculated intratracheally with B. influenzae developed bronch-opneumonia similar to the B. influenza pneumonia in man. All these facts might be, as they concluded, quite favourable to the B. influenzae for its etiologic significance in the pandemic.

In their work, however, there are some points that we cannot approve. Though they describe that the symptoms in produced in the upper respiratory tract of monkeys would be similar to those of the human influenza, we know neither clinically nor histologically pathognomonic signs of the uncomplicated influenza, and so it would possibly too rash to identify these symptoms in monkeys with those of genuine influenza, and to establish the etiologic significance of B. influenzae in the uncomplicated influenza.

Moreover from the fact, that intratracheal inoculation and in some cases the inoculation of the upper respiratory tract with B. influenzae in monkeys could reproduce bronchopneumonia similar to B. influenzae pneumonia in man, they have concluded that the real cause of influenza pneumonia, that is the pneumonia following influenza, must be attributed to B. influenzae. But this is not right. The B. influenzae pneumonia and influenza pneumonia are not synonyms. According

to statistical observations of some authors, namely MacCallum and Fahrig, it is obvious that pneumonia caused by influenza bacillus is not identical with influenza pneumonia, but takes only a small part of the latter. Although B. influenzae may be an etiologic factor in the B. influenzae pneumonia, it can not be the essential causative agent of influenza pneumonia at all.

Further they insist upon the fact that histological changes of B. influenzae pneumonia developed in monkeys correspond with the findings in B. influenzae pneumonia in man. So far as mentioned they may be possibly right, but further to establish an etiologic rôle of B. influenzae pneumonia alone, but in influenza pneumonia as they did, it is lacking in data ; the same histological changes as in human influenza pneumonia should be found in experimental pneumonia in monkeys. The fact is, however, quite on the contrary. Multifariousness which is both macroscopically and histologically common in human influenza pneumonia, is never met with in experimental B. influenzae pneumonia in monkeys. Hemorrhages may often be found in the lattar, but not in such a degree as in man. Necrosis, purulent bronchiolitis and interstitial putrification which are often seen in influenza pneumonia, are not to find. Proliferative changes or metamorphosis into stratified epithelium of the epithelial lining of bronchioles are not the case in monkeys.

Truely Blake and Cecil's experiments show that the B. influenzae may be an etiologic factor in B. influenzae pneumonia in man, but are not sufficient enough to lead them to the conclusion that the same bacillus can cause uncomplicated influenza and influenza pneumonia. The following experiments will fully justify what we have criticized on the conlusions made by Blake and Cecil.

Preliminary Experiment.

A virulent strain "Kuno" was employed in the following experiments. It was cultivated on Levinthal's medium in plate and before application passed successivcly through the peritoneal cavity of a series of twenty five mice followed by a series of thirteen monkeys for the purpose of raising its virulence. At the end of animal passages the original lethal dose for mouse (death in 46 hours with 0,5 mg.) was so much reduced, as to be able to kill the animal in 13 hours with 0,1 mg. of the bacilli.

The findings in passage monkeys are worthy of special mention. When inoculated with larger dose they showed prostration or even collapse, and death resulted in seven of them. The histological observation revealed changes due to an extreme intoxication. This will be of much interest compared with the results obtained in monkeys which received the inoculation in respiratory tract.

In the site of inoculation, the peritoneal cavity, developes an exudative peritonitis. In bronchi and bronchioles the walls and adjacent tissues show constantly inflammative cellular exsudation of a moderate degree, though in the mucous membrane the changes are very slight and inconstant. In four cases

karyorrhexis was found in peribronchial lymphfollicles. It is remarkable that the alveoli of the lung showed almost no sign of inflammation, except a slight cellular infiltration in alveolarsepta, and in some part of the lung we could detect small regions of atelectases and emphysematous dilatation. As to the changes of heart muscle diffuse fatty degeneration was found in three, and waxy degeneration in one case, and the nuclei of muscle fibres present various kinds of degeneration. The liver is congested and in two cases an infiltration of polymorphunclear leucocytes in lobuli was observed. Parenchymatous cells were swollen generally and fatty degenration or necrosis take place very often. The nuclei of liver cells showed also various degree of degenerative change. In five cases we observed deposition of bile pigment. In one case a patch of necrosis was found in pancreas. In the kidney the convoluted portion of tubles was swollen constantly and fell in necrotic deterioration accompanied with degeneration of nuclei. The spleen is congested and Malpighian bodies are enlarged very often with distinct germinal centre. Karyorrhexis in the follicles was recognized in three, and deposition of hyaline substance in two cases. Lymphglands showed no remarkable change. In adrenals were found congestion, small hemorrhages, degenerative changes of nuclei and slight infiltration of polymorphonuclear leucocytes in its cortex.

After animal passages the germ was introduced into the nasopharyngeal cavity or injected intratracheally in rhesus or fuscatus monkeys. The inoculation material used was infected peritoneal fluid of passage monkeys or pure cultures from it in broth. In some cases nasal secretion rich in influenza bacilli was diluted with broth and employed. Besides these, sterilized material was used after heating at 56°C. for one hour as control experiments.

Experiment I.

Intratracheal Injection of B. influenzae.

Let us summarize the findings in five monkeys in this experiment. There is no difference between both series, no matter whether they were inoculated with living or killed bacilli. No sudden onset of symptoms, but after one or two days comes the malaise, which gradually increasing accompanies loss of appetite and prostration, of which the latter was most conspicuous. Rise of temperature, however, was never noticed in all animals, on the contrary often falling subnormally. No sign of coughing and dyspnea. In twenty hours or so after injection leucopenia becomes pronounced, which continues two or three days diminishing to $\frac{1}{2}$ or $\frac{1}{3}$ of the initial count. It is caused mainly by the decrease of neutrophilic leucocytes. On the second days of inoculation we have observed a temporary increase of neutrophilic leucocytes with rod-shaped nuclei, occasionally together with a small number of detached endothelial cells and Türk's so-called "Reizungsform."

Histologically also nothing differ between the findings in the animals

inoculated with living bacilli and those with heated material. In the bronchi and bronchioles are observed more or less contraction, seldom dilatation, and desquamation of epithelial cells, occasionally hemorrhage, defect of epithelium in more or less extent. Inflammative cell infiltrations in the walls and peribronchial tissues are almost constant findings. These changes are much more intensive in bronchioles than in bronchi, and much more pronounced in bronchial wall and peribronchial tissue than on the side of mucous membrane, often accompanied with perivascular cell infiltration. In the lung the principal changes are found in the interstitium, the interalveolar septa being infiltrated constantly with polymorphonuclear leucocytes and thickened. The alveoli show atelectasis or emphysematous dilatation and hemorrhage is very seldom and only to the small extent. The heart is commonly not so much affected, only in one case is found a serofibrinous pericarditis. In the liver the degenerative changes of parenchymatous cells are slight, in two cases spotted or periacinous infiltration of fatt, and fat infiltration of Kupffer's stellate cells too. In the kidney the epithelium of the convoluted portion of the tubules fell in all cases into intensive regressive metamorphosis, showing swelling, desquamation and destruction of epithelial cells and degeneration also of nuclei. Fatty degeneration was observed in two cases. The spleen is congested, the Malpighian body has in some cases distinct, and in other, indistinct germinal centre. Karyorrhexis and deposition of hyaline substance in the centre of the body were observed in one case. The lipoid content of the adrenals was variable, in two cases a slight infiltration of polymorphonuclear leucocytes in the cortex. The skeletal muscles show very often the waxy degeneration, especially in diaphragm and M. rectus abdominis, but rarely in M. intercostalis and muscles of the thigh; fatty degeneration was found in one case.

From the histological observation above mentioned it has been confirmed that in the lung the inflammative process of a certain degree is always limited to the interstitium, while the parenchym is free from marked changes. Degenerative lesions in other organs are, though not so intensive; qualitatively much the same as those of passage animals.

Experiment II.

Inoculation into the upper respiratory tract of B. influenzae.

Of three monkeys which had been inoculated into the upper respiratory tract with B. influenzae developed two animals slight regional disturbances, slight nasal secretion and coughing two days after inoculation, but without fever and other general symtoms. A slight temporary increase of neutrophilic leucocytes is noticed in four to twenty hours after inoculation, gradually returning to, or even below the normal count; but high degree of leucopenia is not the case. Leucocytes with rod-shaped nuclei, detached endothelial cells or Türk's "Reizungsform" are never encountered. Anatomical findings are as well qualitatively as quantitatively

much the same as those of the monkeys subjected to the intratracheal injection, except hyperemia and mucous secretion of the inferior turbinal on both sides and much slighter lesions of the lung.

Discussion.

Monkeys which received the inoculation of B. influenzae into the upper respiratory tract, developed only a slight catarrh of the nasal cavity, and those symptoms which are so common in the human influenza, such as the sudden onset of the disease, rapid rise of temperature and severe prostration, were never observed. So we shall not be justified, when we assert that we have produced in monkeys a disease similar to the human influenza.

Even if through the intratracheal injection, clinically, leucopenia and extreme prostration, and histologically, catarrhal bronchitis, bronchiolitis and inflammative cell infiltrations of the walls and peribronchial tissues were produced, the typical influenza pneumonia with hemorrhage and purulent bronchiolitis etc. was never obtained.

Thus in the two series of inoculation experiment local lesions in the respiratory organ were both clinically and anatomically not very distinct, but degenerative changes in other internal organs were on the contrary much more pronounced, and though they were not so intensive as in passage animals, it was almost impossible to detect any qualitative difference in histological findings between the monkeys of inoculation experiments and passage animals which died of an extreme intoxication. Moreover it must be noticed that the anatomical changes caused by the inoculation of killed bacilli differed in no essential respect from those caused by the living germ. On the other hand, in spite of marked changes, the examination of the distribution of injected bacilli in all organs by autopsies gave always negative results, except one case, in which owing to the retrotracheal escape of the injected material a serofibrinous pericarditis ensued and cultures of the bacillus were recovered from the heart blood, the pericardial and the peritoneal fluid. All these facts lead us reasonably to the assumption that the lesions described above are due to the toxic product of the B. influenzae. Recently McIntosh who had paid much attention to the toxin of the Pfeiffer's bacillus, reported that this toxin could make the animal fall into a rapid collapse and when with a larger dose, death also resulted and hemorrhagic broncho-pneumonia could be produced; the essential changes of influenza, he concluded, were to be attributed entirely to the toxin of the bacillus influenzae. His conclusions, however, do not correspond in all respects to the facts obtained by us.

Conclusion.

1) By way of the intratracheal injection of B. influenzae, which had been raised in virulence by successive passages of the peritoneal cavity through mice

and monkeys, neither typical influenza pneumonia nor bacillus influenzae pneumonia could be produced in monkeys.

2) Though a slight nasal catarrh was produced by the inoculation of influenza bacilli into the upper respiratory tract, it is not yet sufficient enough to conclude that a disease similar to the huamn influenza developed experimentally in monkeys.

3) In short, the re-examination of Blake and Cecil's experiments resulted negative. No sufficient proof to conclude B. influenzae as the causative agent of influenza could be obtained from our experiments.

The Bacteriological Examination of Sputa in searching for Pfeiffer's B. influenzae and other as a possible factor in the Etiology of Epidemic Influenza.

By

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(From the Influenza Research Committee of the Institute.)

A bacteriological examination of sputa or throat swabs from influenza patients as well as those from healthy people and from non-influenza patients, which had been carried out by Matsunami, Kunisaki and Nakamura (1) in 1920, in searching for Pfeiffer's B. influenzae, Fraenkel's Pneumococcus and streptococcus haemolyticus as a possible factor in the etiology of epidemic influenza, has been continued by us with materials obtained in 1921. Materials from influenza patients were collected during the spring epidemic (from January to February), and those of non-influenza people were obtained during non-epidemic season (from April to December) of the year.

165 sputa from patients suffered from influenza, 79 throat swabs of healthy peoples, 194 sputa from patients of pulmonary tuberculosis and 16 sputa of other diseases were subjected for the examination, throat swabs were taken from pharynx, larynx or from tonsils. For cultivating Pfeiffer's bacilli, Levienthal's media (2) and for pneumococcus and for streptococcus horse-blood-agar plates were employed. The reaction of the media were adjusted to lakmus neutral or to +4 by using phenolphthalein. After 24 hours incubation at 37°C suspected colonies were isolated and microscopically and biologically examined. The Pfeiffer's bacillus was grown as a transparent, dew drop like colonies on Levinthal's media and it was negative to Gram's stain and incapable of allowing growth on ordinary agar in the absence of hemoglobin.

The Pneumococcus, when it grows on blood-agar media, manifested changes of hemoglobin into metohemoglobin as to turn the color of the media to greenly black. The colonies are not always alike depending upon variation of the type, but as a rule, it grows as a flatt, small drop showing excavation in the center and thus takes biscuit form, while those belonge to the third type are mucinous and rather large in size and conflict each other. On microscopical examination they are diplococcus with capsel and positive to Gram's stain. The cocci must

be solved by the addition of bile and it ought to ferment inulin. The type of the pneumococci were not examined.

As for the streptococcus, the isolated colonies were determined by a simple microscopical examination, and the specimen which manifested hemolysis on blood-agar-media were recorded as a hemolytic type.

1) Results obtained on the investigation of 165 sputa from influenza patients may be summarised as follows:

Case	Pfeiffer's B.	Pneumococcus	Staphylococcus	Streptoc. haemolyticus	do, non-haemolyticus
165	50	39	82	52	19
Percentage	30,3%	23,7%	50,3%	31,4%	11,5%

The percentage of the propagation of the bacilli appears to be varried relatively in wide limits depending upon the sources of materials and the Pfeiffer's bacilli were found more frequently at the beginning of the epidemic, while the hemolytic streptococci were more abundant in the materials obtained at the end of the epidemic. The marked difference in frequency in the propagation the bacilli depending on appearances of sputa could not been observed except that the staphylococcus were cultivated rather frequently from purulent sputa. Either, the stage of the disease was not an important factor to alter the percentag. Out of 15 patients, who were complicated with broncho-pneumonia, a practically equal ratio to that obtained in the case of non-complicated patients has been cultivated. But, the results of a single examination, as above mentioned, is not satisfactory to draw decided conclusion; because, on repeated examination of different sputa from 16 cases, the bacilli have been found more frequently in proportion.

2) Out of 82 throat swabs from healthy people examined, Pfeiffer's bacilli were positive on 26,8%, pneumococcus on 21,9%, streptococcus haemolyticus on 31,7%, streptococcus non-haemolyticus on 10,5% and staphylococci on 71,9%. Thus, the percentage of the Pfeiffer's bacillus was a little lower than that of influenza patients, but it should be considered as practically the same in proportion when 30,3% of the former case were compared with 26,8% of the latter, there is evidence that the Pfeiffer's bacilli can parasite on healthy people as an a-virulent saprophytes, because, in one of our cases, the bacilli have been found almost continously for 6 months (examined on 5 different occasion) and sera from the same individum contained specific antibodies (agglutinin and complement-fixing body) for the Pfeiffer's bacilli.

3) Sputa from 104 pulmonary tuberculosis obtained in the non-epidemic season, out of which tubercle bacilli were found in about 70%, were subjected for the similar investigations as before. Results are shown by the following table:

	Pfeiffer's B.	Pneumococcus	Streptococcus haemolyticus.
No. of cases examined	178	168	130
Bacillus was found in	35,4%	23,8%	21,5%

4) The similar examination has also undertaken with 18 cases of non-influenza patients, comprising sputa from patients suffered from typhoid fever, from pleurisy, diabetes mellitus, peritonitis, bronchitis and meningitis during non-epidemic period, gave nearly the similar results as in the cases of influenza patients already described. Therefore, no distinct differences in proportion of Pfeiffer's bacillus and others to be found in the case of influenza patients and those of non-influenza patients can be shown by our experiments.

The results obtained on the similar investigation by the previous investigators are varried in wide limits; thus, A. Tanaka (3) states that the Pfeiffer's bacillus was isolated on 86,9% from materials of influenza patients, where the bacillus was positive on 55,0% from those taken from patients suffered from lung tuberculosis, and on 66,6% from healthy people during non-epidemic season.

According to Dwinell (4), out of 488 materials from influenza patients examined, streptococcus haemolyticus was found in 31,8% and Pfeiffer's bacillus in 23,4%; and out of 51 sputa from pulmonary tuberculosis the former was positive to 30% and the latter to 39%. Levin's results on investigating 554 cases of influenza patients, Pfeiffer's bacilli were shown only in 1,26%, but hemolytic streptococci were positive to 91,3% the similar results have been obtained by Rohenberg (6). But Hirsch and Kinney (7), obtained similar results with that of Dwinell and ours.

Hence, therefore, neither Pfeiffer's bacillus, pneumococcus nor the hemolytic streptococcus can be a possible factor in the etiology of epidemic influenza. As they do found relative frequently in the laryngo-pharyngeal secrets of influenza patients, but they are found practically equal in percentage in those from healthy people during non-epidemic period as well as in those of non-influenza patients.

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(*T. T.*)

The Pfeiffer's Bacillus in Sputum of Tuberculous Patients and its immunological Investigation.

By

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and
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The presence of Pfeiffer's bacillus is often proved not only in the influenza patients, but in the diseases which are attended by the pathological change in the upper part of respiratory tract, especially exanthematic diseases, and in sputum and lung cavities of tuberculous patients. The positivity of finding is said to be great in and after the prevalence of influenza, and it decreases with the subsidence of the epidemic. The references to the tuberculous patients are as follows :

Author	Number of Patients examined	Number of positiv cases Pfeiffer's bacillus	Percentage
Klienberger	14	7	50%
Wollstein	24	8	33%
Wohlwill	73	10	22%
Scheller 1906–07 Winter	29	10	33%
,, 1907–08 Winter	105	10	10%
,, 1908 Summer	90	3	3.3%
,, 1908–08 Winter	90	0	0%
Kerschensteiner	35	5	14.3%
W. G. Dwinell	57	34	59%
Leichtentritt	228	58	25%
Ishihara	38	21	55%
Nakamura	10	3	33%
Kajiki sever	12	6	50%
Kajiki slight	78	13	17%
Tanaka 1920 December	22	13	59.5%
,, 1921 April	13	6	46%
,, 1921 October	11	6	54.5%

With regard to pathogenity of Pfeiffer's bacillus for tuberculous patients, some say it is a case of complication, and others say it is simply a parasitic. Discussing clinically the relation between influenza and lung tuberculosis, Burns Anderson, Andrew, Peters, Much and Ulrici Leichtweiss and Hyek say that influenza aggravates lung tuberculosis. But Deiss Curschmann, Kiefer Otto, Creischer assert that tuberculosis undergoes little influence from influenza. We have made our investigation on the positivity of finding Pfeiffer's bacillus in the sputum of tuberculous patients and its immunological relations at the time when there was no prevalence of influenza.

The method of examination.

In order to remove various parasitic bacilli on the pharynx, we washed the sputum three times with physiological salt solution. A small piece of sputum is smeared and cultured on Revinthal plate, and horse blood agar plate. At the same time smeared, stained specimens are made. In majority of cases, tubercle bacillus was proved.

(1) The time of examination.

In the period from July, 1921, to January 1922, the time in which there was no prevalence of influenza, the examination was made of 150 in patients of Tokyo Tuberculous Sanatorium.

Table I.

Number of Patients	Number of Positive cases of Pfeiffer's bacillus	Percentage
150	51 (### 9, ## 8, + 24)	34%

Pureculture of P. B. ## Many colonies + A few colonies

(2) The relation between stage of disease and positivity of finding Pfeiffer's bacillus.

(The stage of disease is from Turban-Gerhardt's classification.)

Table II.

The comparative incidence of influenza bacilli at various stages of the disease.

Number of Patients	The stages of disease	Number of Patients	Number of Positive Cases of Pfeiffer's bacillus	Percentage
134	I	28	7	25%
	II	21	3	14%
	III	85	39	46%

We have found remarkably high percentage at the last stage of disease.

(3) The relation between Pfeiffer's bacillus and mortality.

Table III.

Positivity of finding of P. B. and mortality of patients.

		Mortality	Percentage
Number of patients carrying P. B.	49	24	49%
Number of patients not carring P. B	83	46	46%
Total number of patients	1500	ca 750	ca 50%

The presence of Pfeiffer's bacillus does not increase mortality.

(4) The positivity of finding Pfeiffer's bacillus in different seasons.

Table IV.

Seasonal incidence of influenza bacilli.

Seasons	No. of Cases	Positive	Percentage
Summer (June — Aug. 1921)	54	24	45%
Autumn (Sept. — Oct. 1921)	73	32	44%
Winter (Dec. 1921 — Jan. 1922)	59	23	39%

Seasons do not make any great difference in the positivity of finding of the bacillus.

(5) The result of repeated examinations.

We have made repeated examination of 30 patients in each of three different seasons, Summer, Autumn and Winter. There were 11 instances in which the bacillus was proved every times, 7 instances in which it was negative every times, 12 instances in which it was positive some times, and negative at other times. So of the same patient we could not get the same result. In 6 instances in which many colonies were seen as in the case of pure culture, the bacillus was proved every time.

If as stated before, Pfeiffer's bacillus could be proved in many cases of tuberculous patients, the presence of the immune body created by Pfeiffer's bacillus in the serum of the patient is an interesting problem. Engel and Kajiki

reported that the serum of tuberculous patient is positive in the complement fixation test with Pfeiffer's bacillus.

Agglutination.

Pfeiffer's bacilli No. 1 which were isolated from an influenza patient in the time of pandemic in 1920, and the same bacilli No. 90 which were obtained from a tuberculous patient in winter of 1921, after 18 hours culture, were emulsified with 0,2% salt solution. This emulsion is made agglutinogen. In this test, it was maintained at 56°C, for 18 hours, watching with naked eye and strictly discriminating the appearance of spontaneouns agglutination. Of 50 cases of tuberculous patients, 15 had an agglutination titre of above 1/320 for antigen No. 1., and 14 had the same agglutination for No. 90. The serum of those in whose sputum the Pfeiffer's bacillus was found, was not necessarily positive. There were some whose serum was negative for their own bacillary antigen.

The result of control experiments was as follows:

Of 12 influenza patients and pneumonia patients, there was only one whose serum had an agglutination titre of above 1/320 for No. 90 and among the rest, some had an agglutination titre of below 1/160, for No. 1 and other 8 strains of Pfeiffer's bacillary antigen. Of 8 pleurisy, arthritis, and anchylostomiasis patients, there were 3 cases which had agglutination titre of 1/80, and the rest were all negative. The serum of 3 tuberculous rabbits and 3 tuberculous guinea-pigs were negative.

The Agglutinin absorption test.

The serum of 20 tuberculous patients is treated with No. 1 according to rule, and then it is centrifuged, and the agglutination test of its supernatant fluid is made the result is as follows:

(1) The agglutinin for No. 1 of the serum which is treated with No. 1 is completely absorbed.

(2) The agglutinin for No. 90 is either the same or a little less than before absorption.

From this result we conclude that the serum of tuberculous patients has several times more agglutinin for Pfeiffer's bacillus than that of influenza patients. Whether it is a specific principal agglutinin for Pfeiffer's bacillus or heterologous agglutinin or normal agglutinin which had considerably increased by tuberculosis is the question which needs further investigations.

The Complement fixation test.

Pfeiffer's bacilli No. 1 and No. 90, after 24 hour's culture were made into autolysate by distilled water. To this 0,85% salt was added. This was taken as antigen. Every time we determined the minimum autoinhibiting amount, and used one third of it. The complement was collected from the serum taken from 5

normal guinea-pigs. For haemolysine, anti-goat haemolytic system was used. Every time we determined the unit of haemolysine and two and a half times as much as of it was used. Over against this experiment, we always had necessary control experiment.

Of 17 tuberculous patients in whose sputum Pfeiffer's bacillus was proved, 10 showed strong positive for No. 1 and 4 strong positive for No. 90. Of 10 patients in whose sputum no bacillus was proved, 7 showed strong positive for No. 1 and 2 showed strong positive for No. 90.

For meningococcus and polyvalent antigen of pneumococci used as control, all showed negative.

This result is more remarkable compared to the same examination made of 20 influenza and pneumonia patients.

The sera of 3 tuberculous rabbits and 3 tuberculous guinea-pigs were all negative.

Thus we see that the serum of tuberculous patients has remarkable complement fixing body for Pfeiffer's bacillary antigen. Whether this result is due to specific antigen or nonspecific antigen is an unsettled question which needs further investigation.

Conclusions.

1) The percentage of the cases in which Pfeiffer's bacillus was proved in the sputum of tuberculous patients in non-epidemic period was 34%.

2) The positivity of finding of bacillus in the last stage of tuberculosis is remarkably greater compared to that of first stage.

3) The presence of Pfeiffer's bacillus in sputum does not much influence prognosis.

4) There is not much difference in the positivity in different seasons.

5) The repeated examinations of the same patient do not give invariable appearance. In 6 instances in which many colonies were seen as in the case of pure culture the bacillus was always proved.

6) The serum of tuberculous patients has more remarkable agglutinin and complement fixing body for Pfeiffer's bacillus compared to the serum of influenza and pneumonia patients.

On the Indol Formation of B. influenzae.

By

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(From the Influenza Research Committee of the Institute.)

So far as we know the problem concerning the indol production of influenza bacillus has much been neglected compared with other biological studies of it. Zipfel has assorted in his report this bacillus to that without indol formation. Marcel Rhein and E. Jordan, however, have proven recently on its ability to form indol in cultures, the former on blood agar plates and the latter in broth with sheep blood.

The author made some experiments in this respect after Jordan's method, but applying several kind of blood and with different degrees of heating. The strains employed in his work were isolated from influenza cases and healthy persons during the pandemic in 1919 and their morphological as well biological characters coincided exactly with Pfeiffer's descriptions.

As culture media were used neutral broth with 5 per cent blood. After heating and filtering divided in tubes and incubated at 37°C for 24 hours to test their sterility.

In testing the indol formation in cultures the author applied those method of Kitasato-Salkowski's and Ehrlich's in connection with Weil and Regal's. The blood added to broth were human blood, those of monkey, horse, ox, sheep, goat, rabbit, guinea-pig and pigeon. The most adequate temperature and duration of heating for growth of influenza bacillus was estimated 90°C for 30 minutes. After 2 hours' heating of occurs no growth of the bacilli in any tube.

Indol reaction in blood- broth cultures.

(Culsure media were heated at 90°C for 30 minutes before use.)

Kind of blood \ period of incubation	18h	24h	2 days	3 days	4 days	5 days	6 days	7 days
man	+—++	++—+++	+++	+++	+++	++—+++	+—++	+
monkey	±—+	+—+++	++—+++	+++	+++	++	+	+
horse	+	++—+++	+++	+++	+++	++	+	+
ox	++	+++	+++	+++	+++	++	+	+
sheep	++—+++	+++	+++	+++	+++	+	+	±—+
goat	±—+	++	+++	+++	+++	++	+	+
rabbit	+—++	++—+++	+++	+++	+++	++	+	+
guinea-pig	+—++	++—+++	+++	+++	+++	++	+	+
pigeon	+	++	+++	+++	+++	++	+	+

Among 34 strains examined by the author 10 gave positive reaction, after 1 to 4 day's incubation at 37°C, and it was detected most distinctly and early in media with sheep blood. The remainder of strains even after 7 days' incubation and with any kind of blood gave no trace of indol at all.

As to the relation between degree of heating of media and indol production, it may be said, from authors experiences, that in those strains which are capable to produce indol the amount of indol increases always hand in hand with the favourability of media to the developement of bacilli, and exposure to 70°–90°C for 30 minutes proved to be most favourable to the growth of the bacilli and consequently to indol production. Un-heated media and those exposed to 60°C for 30 minutes or to 100°C for 10 minutes gave slightly positive reaction but after heating at 100°C for 30 minutes or more the results obtained were always negative. I may say, from above experiments, that substance in blood necessary to the growth of influenza bacillus is utterly destroyed by heating at 100°C for 30 minutes

Though the reaction less intensive than with blood, hemoglobin preparates or washed blood corpuscle may satisfactory used instead of fresh blood. Pepton water in place of broth may also give similar results.

Strains which give positive indol reaction retain this character unimpared even after several month's subcultures.

On the Indol Production of B. influenzae.

By

Dr. **Teido Kunisaki.**

(From the Influenza Research Committee of the Institute.)

That some strains of Pfeiffer's bacillus produce indol is already reported by Marcel Rhein and E. Jordan, a fact which has been ascertained also by Suzuki. Recently Yabe has reported that strains of Pfeiffer's bacillus may be assorted in two groups after indol test, though systematic classification in immunological and morphological way of examinations is impossible among several strains. In his experiment, however, the number of strains examined is not so much and it is lacking in the study of the durability of the character to form indol. Therefore I have carried out the same examinations on many strains to Pfeiffer's bacillus, especially as to the durability of the property and whether they differ according to the strains isolated from pandemic, epidemic as well as interepidemic cases.

Strains examined were 93 in total, i. e. 49 isolated from sputa or naso-pharyngeal secretion of influenza cases in pandemic in 1920; 10, isolated from influenza cases in Tokyo Garrison Hospital and Imperial Guard Artillerp; 15, Isolated from pharyngeal secretion of healthy persons from March to August, 1921, a period during which no influenza epidemic had prevailed; 19, isolated from sputa of phthisics in the Municipal Sanatorium of Tokyo in July to November 1921 in an interepidemic season.

For cultivation of bacilli fluid media were employed; to after Levinthal's prescription horse blood is added weak alkaline pepton water or bouillon, after heating filtered through filter paper or Berkefeld or Reichel filters, divided in tubes and before use incubated for 2 days at 37°C to test their sterility.

To detect indol formation in cultures Ehrlichs test especially ring method and Salkowski's reaction were adopted on those tubes inoculated with B. influenzae which after 2 or 3 days incubation at 37°C revealed a full growth of the same bacilli without any contaminations.

As we are informed of strains of Pfeiffer's bacillus, which isolated from a sputum may differ in their immunological characters, and from our previous experiences those bacilli isolated from a same healthy person on two different occasions may also differ in the ability to produce indol, so I have in my preliminary experiment spread bacilli grown in above media on different blood agar plates and after growth of separate colonies they were taken up arbitrarily in separate tubes and tested on indol formation. Such examination on three

strains has revealed no difference among colonies on the same plate. However the quality of culture media has much influence as wall upon growth of the bacilli as indol production, it must be born in mind that indol test is to determine only in those media which give sufficient growth of the bacilli shown in them.

The results obtained were as follows:

(1) Strains isolated in pandemic in 1920
With indol formation 17
Without „ „ 32 total 49.

(2) Straines isolated from influenza cases in February, 1921.
With indol formation 6
Without „ „ 4 total 10.

(3) Strains isolated from healthy persons in March-August, 1921.
With indol formation 10
Without „ „ 5 total 15.

(4) Strains isolated from sputa of phthisics.
With indol formation 12
Without „ „ 7 total 19.

Thus among 93 strains indol production was detected in in 45 of them. They may be summarised again.

(1) Influenza cases
With indol formation 23
Without „ „ 36 total 59.

(2) Non-influenza cases (phthisics and healthy persons)
With indol formation 22
Without „ „ 12 total 34.

(3) From morbid cases (phthisics and influenza cases)
With indol formation 35
Without „ „ 43 total 78.

As to the changes of distribution of these two groups of bacilli in healthy persons and influenza cases also in different epidemics must be discussed upon further experiments.

The ability to produce indol is maintained through several subcultures on blood agar even after a year and half, vice versa. Passage of bacilli through mice does not effect any change in their original characters. In spite of the fair contrast in indol reaction they never be arranged immunologically as already experienced by Matsunami and Nakamura as well also by Yabe.

Conclusion.

1) B. influenzae may be divided into two groups, i. e. those with and without ability to produce indol.

2) Though the one gives constantly positive reaction on adequate media

the other lack in this character even in media which allow full proliferation.

3) The ability of producing indol is maintained for a long time through frequent subcultures.

4) No definitive difference can be detected as to the occurrence of these two groups among pandemic and epidemic influenza cases, phthisics and healthy persons, as also immunological classification has failed to several authors.

A new method of Classification of Pfeiffer's bacillus by Fermentation of Carbo-hydrates.

By

Dr. **Jiro Nomura.**

(From the Influenza Research Committee of the Institute.)
(Published previously in Japanese in the Japanese Journal of Experimental Medicine, Vol. IV. 1922.)

The description about the biology of Pfeiffer's bacillus was very simple until to-day. It failed to classificate the Pfeiffer's bacillus by serological method (complementfixation and agglutination) notwith-standing utmost-effort of many insvestigators.

Only lately Jordan succeeded to divide into tow groups in this bacillus by Indol-reaction which is produced in culture media of the bacillus. But when we consider the nature of this bacillus, we can not satisfy to divide only 2 groups in it.

Therefore I tried to classificate it after the method of the classification of colon bacillus by fermentation of carbo-hydrates. In the beginning of this work I cultivated the bacillus on the following media (normal-agar filtrated boiled horse blood, carbo-hydrates as indicator lithmus or fuchsin reducted by sodium sulfit). But I could not reach satisfactory result.

By further research I found that the disturbing factors must be the contention of dextrose in bouillon and horse blood (horse blood has 0,1=0,4% glucose). So I tried to take away the glucose from horse blood. For this purpose I examined following three methods.

1) To destroy the glucose of the blood I used the fermentactivity of erytrocytes, but thereby the hemolyse occured always.

So I considered this method is not suitable for this purpose.

2) I used the yeast of beer or Japanese Sake.

3) I dialyzed the boiled horse blood and bouillon. By the dialization the glucose was taken away totally in 5 days, but thereby the dimming developed in bouillon. So I considered this method is not so good as the 2nd method and concluded to use for the blood the dialization, for bouillon the yeast.

The method of preparation of my media.

1) Physiologic saline solution of two fold volume is added to one volume horse blood. The mixture is heated 5 minutes in boiling water bath, being stired meanwhile and then filtered through filter paper.

The filtrate is dialyzed through swine intestine in running water in 5 days and sterilized by 60°C 30 minutes 5 days succesively.

For the test of sugar in this filtrate the phenylhydrazin-reaction was used.

2) Preparation of bouillon.

One loop full of yeast of beer is added to 1 liter normal horse bouillon and incubated by 37°C one week and the mixture was boiled to sterilize.

3) Preparation of agar.

The 3% agar is manufactured from the before described bouillon, witte pepton (1%) and salt (0,5%) and is sterilized by normal method.

4) The before described horse blood filtrate is mixed to this agar by ca. 60°C

5) 10% carbo-hydrate solution in destilled water is filtered through Reichert's filter for the purpose of sterilization.

The prescription of my media.

1)	3,5% neutral agar	60,0
	boiled horse blood filtrat	30,0
	lithmus fluid	10,0
	filtered carbo-hydrat solution	15,0
2)	3,5% neutral agar	70,0
	boiled horse blood filtrat	30,0
	filtred carbo-hydrate	15,0
	saturated fuchsin alcohol solution	0,25
	sodium sulphite	0,2–0,25

Result of fermentation test:

When I cultivated the bacillus of 106 strains on the (1) media, by which the lithmus is used as indicator, only glucose media reddened but other carbohydrates did not redden.

When the bacillus were cultivated on the second media, in which the fuchsin is added as indicator, and incubated 48 hours by 37°C. I could classificate 8 groups in 106 strains of Pfeiffer's bacillus through fermentation test.

A. group (68 strains). fermented dextrose, dextrin, maltose, lactose, starch, inulin.

B. group (19 strains). fermented dextrose, dextrin, maltose, lactose, starch, inulin, galactose.

C. group (6 strains). fermented maltose, glucose, lactose, galactose, starch, inulin.

D. group (6 strains). fermented maltose, glucose, lactose, starch, inulin.

E. group (2 strains). fermented dextrin, glukose, lactose, starch, inulin.

F. group (1 strains). fermented glucose, lactose, galactose, starch, inulin.

G. group (1 strains). fermented dextrin, maltose, glucose, starch, inulin.

H. group (1 stains). fermented dextrin, maltose, mannit, glucose, lactose, starch, inulin.

Notice;

1) I studied the influence of the duration after isolation of bacillus on their fermenting faculty and recognised that there is no disturbing influence.

2) The method of division through indol test did not coincide with my method.

On the place of production of Agglutinin for Pfeiffer's bacillus and its Distribution and local immunity.

By

Dr. **Jiro Nomura.**

(From the Influenza Research Committee of the Institute.)

(Published previously in Japanese in the Japanese Journal of Experimental Medicine, Vol. IV, No. 11, April 1922.)

It is well known fact that the place of production of antigen in the body of animals is different accoding to the kind of antigen. Since the state of agglutination of Pfeiffer's bacillus has the characteristics distinguished from those of other bacilli it might be interesting to make research with regrad to the place of production of agglutinin for Pfeiffer's bacillus. Under this assumption the following experiments were made. When a rabbit was killed slowly by the subcutaneus, intravenous and intraperitoneal injection of this bacillus, the first places in which the presence of agglutinin was proved were the hematopoietic organs, especially spleen and bone marrow. In twenty four hours after the injection of antigen it was clearly proved and at an elapse of twenty eight hours agglutinin was proved in serum, the liquor of peritoneal cavity, lymph, liver, pancreas, adrenal body, cerebro-spinal fluid. With an elapse of ten days I could never prove agglutinin in the leukocyte, lymph-gland, thymus, bile, kidney, cerebrum, cerebelum, medulla oblongata, spinal cord, testicle, ovary, uterus and aqueous humour of eye. After the immunization of the animal by the subcutaneous, intraperitoneal or intravenous injection of a large quantity of Pfeiffer's bacilli, agglutination was positive in the dilution of 1 : 320 to 1 : 640 of the spleen extract, 1 : 320 of bone marrow, 1 : 640 to 1 : 1280 of serum, 1 : 320 of the peritoneal fluid, 1 : 160 of lymph, 1 : 40 to 1 : 80 of the liver, adrenal body, cerebro-spinal fluid, heart muscle and lung, and it was negative in other organs and tissue fluid. This led me to suspect that the production of agglutinin for Pfeiffer's bacillus may have some relation to the hematopoietic organs, especially spleen. After a month if the complete removal of spleen from the rabbit, the subcutaneous, intraperitoneal and intravenous injection of Pfeiffer's bacillus was made. In none of the tissue fluid and organ extract I was able to prove agglutinin. At the elapse of three months ofter the removal of the spleen, agglutinin was not proved as in the former case. When the injection

of Pfeiffer's bacillus was made to the rabbit at the elapse of $7\frac{1}{2}$ months after the removal of its spleen, a remarkable large quantity of agglutinin was proved. The serum agglutinated the bacilli in 1 : 5120, the bone marrow in 1 : 1280 to 1 : 2560, and in the liver, pancreas, adrenal body, cerebro-spinal fluid, heart muscle and lung, agglutination was all positive. This fact shows that the function of the spleen is compensated by other organs. I injected benzol into rabbit in order to cause the functional disturbance of its hematopoietic organ. Then it was immunized with Pfeiffer's bacilli and the production of agglutinin was found remarkably hindered.

In order to find out whether agglutinin is produced immediately by sensitization of Pfeiffer's bacilli or not, the following experiment was made. Pfeiffer's bacilli were injected into the auricular vein of the rabbits. After $1\frac{1}{2}$ and 3 hours from the inoculation, the spleen was removed. In the tissue fluid and organ extract of the rabbit which passed eight days after inoculation, I could not prove agglutinin. On the rabbit whose spleen was removed after 6 hours from the inoculation, the extract ot bone marrow had agglutination titre of 1 : 160, the serum and adrenal body were all positive having title respectively in 1 : 160 and 1 : 80. On the rabbit which has passed 72 hours after the injection of antigen, the serum, bone marrow were positive in 1 : 160 and also positive in other organ.

The excised spleen had an agglutination titre of 1 : 320. From these experiments it would be clear that the spleen taken an important rôle in the production of the agglutinin for Pfeiffer's bacillus. In order to find out the length of period during which the Pfeiffer's bacilli which was instilled into the body of the rabbit is contained in the spleen and blood, 5 c. c. of blood was taken from the spleen and heart of the rabbit after an elapse of three, six and twenty-four hours from the intravenous injection of the bacillus at the ear lobe and then the production of agglutinin was examined. In the gninea-pig into which was injected with the spleen or blood of the rabbit which had passed 3 or 6 hours form the inoculation of antigen, agglutination was positive. In the guinea-pig into which was injected the spleen of the rabbit which had pased 24 hours after the inoculation, agglutination was positive, but the guinea-pig which was inoculated with the blood of the same rabbit, did not show agglutination.

In the second experiment, the question whether or not there exists the local immunity for Pfeiffer's bacilli was investigated and the following result was obtained. In the site of the subcutaneous injection, agglutinin was proved after 48 hours. When intracranial injection of antigen was made, agglutination in the spleen and bone marrow was proved after 3 days from the inoculation. Agglutination was positive in 1 : 80 in the cerebro-spinal fluid. It is quite note worthy. That the extract of the spinal cord had an agglutination titre of 1 : 20. But in the cerebellum and medulla oblongata I could never proved agglutination. Even in the rabbit which passed 4, 6 and 7 days I could prove agglutination of the extract of the spinal cord. Even if we instilled antigen into the aqueous humor of anterior

chamber from other part, we could not prove agglutinin. On the contrary if the antigen was injected directly into the anterior chamber, agglutinin was showed in that part and in other tissues.

An Experiment of the Route of Elimination of the Bacilli by experimental Paratyphoid Infection of rabbits.

By

Dr. **Megumu Arai.**

(From the fourth Section of the Bactrio-Serological Department.
Head of the Section, Dr. Itsuma Takagi.)

It is known that the principal routes by which bacillus of the typhoid group leave the patient's body are intestines (faeces) and urinary apparates (urine). There are beside these typhoid carriers.

In the case of typhoid carriers, the gall bladder is considered as the source of the bacilli which appear in the intestine. But on the part played by each of organs of the urinary tract to eliminate the bacilli with urine, it is still under discussion. Thus, investigations have been carried out on this line by experimental typhoid fever in animals.

Among bacilli belonged to the typhoid group, the Bacillus paratyphosus B which had been passed rabbits several times, were used through out the present work; as, it is the most convenient material for the test, from its virulence and from the duration to be hold in the animal body.

Rabbits weighing from 2000 to 3000 gramms were employed for the experiment.

Among organs of the urinary tract, kidney should, of course, be taken at first in consideration for the study of the cause of urinary carriers of the typhoid infection. Thus, the kidney of the infected animal was principally examined in the present paper in comparison to the investigation of the gall bladder.

(1) The attitude of paratyphoid bacilli in the gall bladder.

Three methods have been given to submit rabbits to the typhoid infection; i. e. (1) injection of the virus into the vein; (2) injection directly into the gall bladder and (3) injection into the bone marrow of the experimental animal. Among these, the last method, given at first by Takaki, were chiefly employed, as it is easiest to carry out and symptoms of infected animals are closely similar to those in the case of human typhoid infection.

On 2 series each consisted of 5 rabbits, which were previously injected with

killed culture of paratyphoid B bacillus, 6 milligramms each of virulent culture of the bacillus were inoculated into the vein or into the bone marrow, respectively to each set of the rabbit. By injecting the virus into the bone marrow (the second series), the loss of appetit and the wasting of the infected animal was not marked, but by intravenous injection distinct emaciation accorded on majority of animals. On 15 th day after inoculating the virus, all animal were killed and examined histologically as well as bacteriologically. On Bacteriological examination, the virus were cultivated successfully from the content of gall bladder of all ten rabbits. At autopsy, the gall bladder is usually distended in size according to the stagnation of bile and fibrineous patches can occasionally be seen on the outer surface of the organ.

The histological changes of the viscus are minor in general; but on animals of the second series, the changes were relatively remarkable than those of the first. Thickning of the wall (till 1, 8 mm.), infiltration of leukocytes, erosion of mucosa or formation of ulcer and dilatation of blood vessels can be demonstrable in almost all cases. The contents (the bile) is thicker, darker and more turbid than normal and sometimes it assumes a milky appearance, owing to the accumulation of small white flocci.

As mentioned above, the specific virus is found as a pure culture from such contents of the gall bladder; but it is very hard to follow the virus in the tissues. In one case, however, a numerous bacilli were demonstrated in the proper membrane of mucosa, epithels of which were desqumated, a few bacilli have also been demonstrated in the lymph cavity and in the blood vessels of submucous membrane. In general, changes on the gall bladder are advanced in the series of the animal into which the virus are injected into the bone marrow, and they are closely similar to those in the case of human typhoid infection. As to whether the usual route of the bacilli invading from the circulating blood into the gall bladder is the capillaries of the viscus, or the bile duct passing through the liver from the portal vein, the powerfull evidence is not given to draw any decided conclution, except that the bacillus can be seen in the lymphatic and venous cavity of the wall of the viscus.

(2) Kidney as a route to eliminate paratyphoid B bacillus.

Of all human typhoid or partyphoid infection, the frequency to pass virus into the urine lies on about 50%. But in the case of typhoid carriers, the faeces is the dominating factor to eliminate the bacilli; thus, in only 7% of all typhoid carriers the bacillus has been said to be found in urine.

Two different considerations are held by the previous investigators to manifest urinary carriers; of which the one hold the view, that the pathological changes of the urinary tracts are due to which the bacillus multiplize continuously; but the other could not show any changes throughout organs of urinary tract.

As stated by Faulhaber, in all three cases of human typhoid, the specific

virus were demonstrated in the small veins and in the capillaries of the cortical substance of the kidney, from the urine of which the bacillus had been cultivated beforehand. And in 2 cases of them, there showed embolism of the bacilli in the tufts of glomerulus, and a few bacilli were also found in Bowman's capsule; although, no reactive changes of the tissues could be observed around such groups of the bacillus. Such embolus of bacilli should be not only a source of eliminating bacilli in urine during typhoid infection. Neufeld and others believe that the pathological changes of the tissues are neccessary to admit to pass the bacilli from circulating blood into the urine, but according to Biedle and Kraus, the bacilli can be filtered through healthy capillaries into the urinary canal.

By experimental infection of rabbits, however, no marked changes of the tissues of the kidney were able to be followed, while abundant bacilli were found in the urine and in the kidneys of such animals.

(3) Production of abscess in Kidneys on experimental animals by paratyphoid infection.

Rabbits were immunized previously with killed culture of paratyphoid B bacillus. The left ureter of such animals were ligated by the method described by Harttung. Four milligrams of virulent paratyphoid bacillus were injected intravenously into the animal immediately after the operation; after 9 days interval, 3 milligrams of the virulent culture were again injected into the bone marrow. On 18 th day after the first injection of virulent culture the rabbits were killed in order to examine the result.

At autopsy, the kidney on the ligated side showed typical pyonephrosis due to the obliteration of ureter and to the infection with typhoid bacilli. The abscess thus formed can be accepted as a "Ausscheidungsabszess" in the sence named by Orth. As, in animals killed in relatively earier stage of the infection, it can easily be seen that the abscesses are initiated from the eliminating focus of the kidney.

Both the artery and vein of the kidney on one side were ligated on the other series of rabbits. All animals were immunized with killed bacillus immediately after operation. With 10 to 25 days interval, 10 milligrams of virulent culture were injected into the bone marrow of such animals. In 4 to 5 th day after the last injection, isolated abscess of the paratyphoid infection has been demonstrated in the medullar substance of the kidney of the opposit side (3 out of 4 cases). The operated kidneys were, of course, undergone to necrosis. It has been stated by Baner and others that, when one side of the kidney is affected, the other one is suffered readily from infection, owing to the overfunction and the debilitation (Debilité renale, martius).

In our case also, the similar explanation can be adopted for the cause of the production of the abscess on the healthy kidney when the other one is affected; but the action of nephrotoxin from the necrotic kidney should also be taken in

consideration. However, the experimental production of the solitary abscess on healthy kidney observed in the last experiment, might be an interesting evidence to the manifestation of urinary typhoid carriers, as the similar abscess can often be observed in the kidney of human typhoid patients.

(*T. T.*)

On the reproducing Paratyphoid B. Infection by Oral introducing in Rabbits and its Immunity.

By

Dr. **Megumu Arai.**

(From the fourth Section of the Bactrio-Serological Department.
Head of the Section, Dr. Itsuma Takagi.)

I have reexamined the reproducing paratyphoid B. infection by oral introducing as Besredka described in following articles " Reproduction des infection paratyphiques et typhiques, sensibilisation au moyen de la bile " and " de la vaccination contre les eats typhoides par la voie buccale ", and will communicate certain new knowledge on this point. With regard to the natural immunity of rabbit against typhoid and paratyphoid-infection, it depends upon the local immunity of intestinal epithelial cells as Besredka described. If the rabbit swallows previously a certain amount of ox bile, and is suffered from catarrh of intestine, then the thus treated rabbit is easy infected by introducing of a large dose of bacilli applicated by the mouth, showing diarrhea and other symptoms, and having the similar pathological appearances in bowels to those of typhoid and paratyphoid infection of man. If such infected animal is survived, its immunity against responsed bacilli is stronger and more permament in compair with those acquired by vaccination.

For the sake of reexamination of the Besredka's result, I used rabbits weighing about 2 k.g. which swallowed antificially 8 c.c. of ox bile once or twice daily and ofter starvation for 24 hours they swallowed again an emulsion of 24 hour old agar culture of paratypoid bacillus in saline solution — 50–60 milligram of bacilli in 6 c.c. of saline solution. The animals suffered from diarrhea caused by bile and were prostrated, no lively at all and were dead after 2–5 day. On autopsy, there were hyperemia and hemorrhage on the mucus membrane of duodenum, and intestine. But no microorganisms introduced were seen.

What is the cause? In order to determin this phenomenon, I mixed a certain amount of paratyphoid bacilli with the gastric juice of rabbit and put this mixture for various time, 5, 10, 20 and 30 minutes, and then cultivated the baccilli from this mixture by ordinary way. Some of them were steril already in 15 minutes and the latest in 45. The sterilising power might depend upon the acid content of gastric juice. Therefore I neutralised the gastric acid with bicarbonate

soda and then put in thus made neutral fluid a certain amount of bacilli, in order to repeat the experiment above described. I could find the bacilli survived over 24 hours.

For the sake of examination of this fact in living body, I tried 2 series of experiments, (1) introducing in rabbits a large dose of paratyphoid B bacilli by the mouth (2) introducing the same dose of bacilli after neutralization of the gastric juice with 1,0–1,5 gram of bicarbonate soda. After 24 hours the experimental animals were killed. The corresponding bacilli were found in a large number from duodenum, and relatively a few from small intestine of the second series and none from the first. From this fact I might be able to conclude that the sterilising power of the rabbit's stomach to bacilli depends upon the content of acid. Whereas the reproducing paratyphoid B infection takes place not only by sensitizing the bacilli with ox bile as Besredka found, but also by previously neutralization of gastric acid with bicarbonate soda.

In the second series I found a certain pathological changes on the intestine and also on the gall-bladder.

Oral vaccination.

The problem of vaccination by the mouth was studied up to the present timy by many scholars, and Jötten, Minami and others were able to demonstrate in the serum agglutinin, bacteriolysin and other immune bodies responsed to the bacilli used. I tried to study this point in following manner,

(1) By introducing into rabbits an emulsion of paratyphoid B bacilli in saline solution heated for half an hour at 60°C., (2) by introducing an emulsion the same amount of living ones and (3) in the last series by introducing the same amount of killed or living bacilli after the neutralization of gastric acid with bicarbonate soda. After elapse of 15 days the serum of all amounts were examined from the immunological stand point. In each series the titer of agglutination was found in 1 : 500, Besredka found, if the experimental animal was introduced by the mouth a certain amount of living or killed bacilli after the sensibilization with ox bile, the animal obtained a local immunity of a sure and permament nature on the intestinal mucous membrane, although the animal possessed no antibody in its serum. On account of the disadvantage of ox bile described above, I used the toxin of dysentery bacilli in a sublethal dose by the intravenous injection into the rabbit and after elapse of three days I introduced a large number of living bacilli with previously neutralization of gastric content. By this way the death rate of experimental animals was lower than that of the infected animals sensitized by ox bile. The titer of agglutination was 1 : 1000. Two or three weeks later the thus treated animals were inoculated intravenously with a supralethal dose of bacilli and they showed much more resistant against infection than those of the following control experiment : (a) vaccinated animals

by the vein, (b) by the mouth with a large dose of killed bacilli, (c) by the mouth with a large amount of living bacilli and (d) by the mouth with a large number of living bacilli after neutralization of gastric juice.

Generally speaking, the content of the upper part of the intestine, namely of duodenum or jejunum is free from microorganism that is to say steril, which takes place by the action of a so-called either autosterilization or autodisinfection. What is the cause of it? I wanted to clear up the origin of this property of the intestine, I made the following experiments.

(1) The rabbits were inoculated intravenously 3 milligram of living paratyphoid B bacilli and were killed after elapse of eight days in order to examine the bacilli, a large number of bacilli were found in bile and in the content of duodenum, a few in that of small intestine.

(2) The previously vaccinated animals by the vein were injected intravenously with living bacilli and were killed after elapse of ten days, having a large number of bacilli in gall-bladder and in duodenal content and a few in small intestine.

(3) The previously vaccinated animals with a large dose of vaccine by the mouth were inoculated intravenously with living bacilli 15 days after vaccination and on the autopsy after 8 days the content of gall-bladder showed a large amount and duodenal and intestinal content a few of bacilli.

(4) The previously vaccinated animals with living bacilli by the mouth were injected intravenously with living bacilli 15 days after vaccination and on the autopsy after 8 days one found a large number of bacilli in gall-bladder and duodenum and relatively few in the small intestine.

(5) The rabbits vaccinated repeatedly with a large dose of the living bacilli through the mouth after neutralization of gastric juice were instilled intravenously with living bacilli and the autopsycal examination after 8 days showed a large amount of bacilli only in the gall-bladder and none in the intestine.

(6) The rabbits vaccinated with dysentery toxin and treated with the same manner as that of No. 5 showed also the same result.

From the above described experimental results I may be able to conclude that if the rabbit is treated once with living bacilli acting on the duodenal surface, it is afterward to have no bacilli on the duodenal content, even if the bacilli in gall-bladder were flown out into the duodenum, kill them immediately.

On the contrary, the extract of duodenal contents and its mucous memberane with saline salution had no property to kill the paratyphoid B bacilli. Hence the autosterilization of duodenal mucous memberane depends upon the vital force of its cells.

Expedition for prevention of Dysentery prevailed in a small Island : Toshima.

By

Dr. **Tsunetomi Yamada**, Dr. **Zenjuro Inouye**
and
Ryoji Kakinuma.

The island, Toshima, situates in the Pacific Ocean, about eighty-five miles far from Tokyo and contains 365 inhabitants.

In the last spring, April and May, a severe epidemy of dysentery created by one carrier from Tokyo took place among these poor people. We made an expedition to this island on the 11th May by the Home Government order, in order to determine what is the cause of the epidemic disease and then to apply the preventive measurement.

From the bacteriological and serological point of view, we were able soon to settle the virus of epidemy as bacillus dysenteriae Shiga.

For the prevention, we used the vaccination applied by simultan method on nearly whole people and suggested them other suitable measurement in preventive view, in all daily necessities, especially in food, drink water and desinfection of faeces and urine etc.

Fortunately the prevalence ceased gradually until the end of June, with 36 patients and among them 2 dead cases.

On two Strains of Cholera vibrio showing no motillity.

By

Dr. **Keizo Nobechi.**

(From the third Section of the Bacterio-Serological Department.
Head of the Section. Dr Takeo Tamiya)

In spring of 1921, the author found the fact, that two strains, named "Takahashi" and "Koch", among over 80 stock cultures stored in the 3rd. laboratory of the 3rd. section in our institute, revealed no motility. In spite of the efforts offered in various ways to confirm it, the motility has never been recognized on them in 18 months thereafter. During this period, transplantations have been carried out monthly, and the immotility has proven to be inheritable on them.

"Takahashi" is a strain isolated in 1917. A same strain has been stored separately among the stocks of the vaccine preparation room of our institute, this strain does not show any motility also. "Koch" is a strain, given to our stock in 1920 from Dr. Kabeshima. This strain is said to be a strain called "70" formerly in Koth institute in Berlin.

Cholera vibrio has, as a character, the most active motility of all bactera. Even such old strains as "Hamburg", over 30 years old, and "El Tor", nearly 20 years old reveal still typical movement on their young cultures. The loss of motility is a marked feature for a cholera virus.

The Author examined at first the two immotile strains in the microscopical, cultural, biological as well as immunological characters to ascertain whether they are true cholera strains; as they proved to be real cholera virus in those respects, the reversing experiments, to make it motile, was carried out in the second place.

Firtsch[1] noted at first on immotile variant of Finkler Prior's vibrio on gelatine, Fürst[2] studied on the same variant and reported such a variant formed dry dark colonies on agar. Olsson[3] reported on the artificially produced immotile variants of the vibrio cholerae, which formed dry and dark colonies on agar plates, and could return to moving ones in 3–5 weeks on slant agar. Violle[4] gave a simple note on existence of immotile strains but without descriptions on their colonies and other characters.

Examinations.

Morphologically, "Takahashi" is short and "Koch" is long, but both are curved as in the case with typical vibrios. No flagella was recognized on both of them. Movement was examined repeatedly in various ways, but only Brownian movement was to be seen.

Colonies on agar plates are typical for vibrio. They have high alkali torelance.

Proteolytic fermentation and cholera red reaction were proven to be positive. Behaviours to various sugars are same with other typical cultures, having a single exception: i.e. "Koch" fails almost to ferment saccharose, therefore it forms but slightly pink colonies on Aronson's medium,[5] while others deep red ones on it. "Koch" reveals a strong hemolytic activity, but less intensive than "Eltor 4", "Takahashi" shows a weaker hemolytic power. They have moderate virulence against guinea pigs as cholera vibrio, and the virulence increases by guinea pig passages.

Immunological examinations.

The agglutinability and the agglutinogenetic character of the immotile strains show that they are strains of the main type of Kabeshima[6]. In agglutinin absorption test, they absorb only the agglutinins for the main type.

The immotile strains are agglutinated slower than the motile. Immediately after the incubation of 1 hour at 37°C., agglutination of the immotile strains is markedly weaker than that of the motile, and the floccules showed by the former are far finer than those by the latter. But after standing for 24 hours at the room temperature, both attain the same titer. The formalinated emulsions of the motils strains behave entirely same as the immotile strains. The above mentioned difference in agglutination is based therefore merely upon the motility.

In the Pfeiffer reaction test, they show almost the same result with "Inaba", a strain of the main type.

Thus except the absense of motility, they fulfill all the data for a typical cholera vibrio. the lack in ability to ferment saccharose in "Koch" is but trifling, compared with the satisfaction of all other data, and can be disregarded.

Reversion experiment.

That repeated transplantation in broth make the masked motility of bacteria active, is reported by some authors. György determined the motility of virus after the performance of 20 bouillon passages, transplanting twice daily: the author applied this method on the immotile strains as a reverion experiment, without being able to recover the motility at all.

Passage experiments with guinea pigs were performed, introducing intraperitoneally, with "Koch" up to the 16th. and with "Takahashi" up to the 14th. generation. No reversion in motility has resulted subsequently. But the

peritoneal cholera is at any rate not the normal state for the cholera vibrio.

The intestinal cholera and the gall bladder infection were tried for the same purpose in rabbits. The virus recovered from gall bladder and intestine showed equally no tendency to show motility.

Motility was not regained by staying in bouillon, pepton water, serum and gelatine, during one month.

Old cultures on agar over 4 months also could not produce any moving elements.

Discussion.

The immotile strains observed by author have moderate virulence against guinea pigs, and gain a virulence increase by animal passage. Possibly it may be noxious also for man; and, increasing its virulence by human passage it may cause an epidemic.

If an immotile strain is found from human case, it may offer confusion in the diagnosis of the first case.

The immotile variant forming a dark dry colony as observed by Olsson is apt to be missed in isolation, while the immortile strain forming normal colonies as mentioned by the author may be isolated, and cause trouble in diagnosis.

Though the variant of Olsson returned to a motile after standing 3–5 weeks on agar, the variants of the author do not return even after 4 months, they may be very hardly reversible variants, or true irreversible mutants in the sense of de Vries.

Conclusion.

1) Immotility of the two strains in question have been proven to be inheritable, at least, for 18 months.

2) Except the immotility, they are typical cholera strains, in morphological, cultural as well immunological respects.

3) All reversion experiments in their motility ended in failure.

4) "Koch" fails to ferment saccharose, and its colonies on Aronson's elective medium are not stained so intensively red as in the case with other strains.

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On the Cholera Strains encountered in Japan in 1921.

By

Dr. **Keizo Nobechi** and **Tomoji Ishikawa.**

(From the third Section of the Bacterio-Serological Department.
Head of the Section, Dr. Takeo Tamiya)

In 1921 two slight epidemics of cholera happened in Japan. The first one occured in early summer in Kanagawa Prefecture, with a case also in Tokyo. It consisted of only three patients and two carriers. The authors could obtain the strains from all of them. They are called "Inaba", "Sano", "Wada", "Chokichi" and "Suga". In autamn another epidemic arose in the district near Nagasaki, and three strains were collected from those cases, namely "Ogawa", "Hirahara" and "Kubo". Moreover, three carriers were brought from Shanghai, China, and detained in the quarantine station at Hikoshima of the port Moji; three strains from those carriers could be obtained; they are called "Hikoshima 1", "Hikoshima 2" and "Hikoshima 3". Another patient who was dying, was brought to Tsingtao from Shanghai also, and a strain from that case, "Tsuda", was sent to us from the Tsingtao quarantine station.

Thus 12 strains were furnished to our study, it was our task to select out the varieties logically fit for preparation of the vaccine and anticholera serum.

Microscopical.

All strains had typical shape of vibrio. They revealed all vigorous motility and those, on which flagella staining was performed, possessed single flagella on one pole.

Cultural.

They showed high alkali torelance and grew copiously on Dieudonnè's and Aronson's media.

Several strains revealed varied colony forms on agar plates. A transparent colony and a turbid one, as noted by Baerthlein,[1] isolated from "Suga" strain proved to vary in its hemotoxin production, but showed no immunological difference. A dark dry colony as noted first by Eisenberg[2] was also encountered not infrequently; a dry dark colony isolated from "Suga" strain had a strong tendency

to spontaneous agglutination as that reported by Puntoni,[3] and all the varied colonies were reversible to the typical one.

Biological.

All strains gave positive cholera red reaction, showed proteolysis on Loeffler's serum and gelatine, revealed same fermentative action on various sugars as the other typical strains, and did not clot milk.

They showed moderate and almost the same grade of hemotoxin production, though weaker than "El Tor 4."

The virulence of the strains were tested with emulsion in bouillon from the 24 hours old slant agar culture. They showed varied virulence ranging from 1/2 to 1/40 loopfull against guinea pigs weighing 200–250 gr. "Wada" showing primary virulence of 1/40 loopfull, was kept long in high virulence by animal passage and used as a test virus for standarization of anticholera serum.

Immunological.

Dr. Takaki[4] reported on four immunologically varied types, but they had died away and we cannot compare the new strains with his types. The main type and the varied type reported by Dr. Kabeshima[5] have been accepted also by other authors. Rabbit immune sera were prepared by the representatives of the new strains and a cross agglutination test was carried out. A typical strain of the main type called "Hamburg" and such of the varied type called "Giran 2" and their immune sera were used as control. Table 1 shows the result of the test.

The serum "Hamburg" divides the new strains into two groups: nine strains from "Inaba" to "Tsuda" in the table react high as the strain "Hamburg", while three strains "Ogawa" to "Kubo" low as "Giran". And further, the members of the former group are divided into two groups by the serum "Giran": strains "Inaba"-"Suga" react low as the strain "Hamburg"; while strains "Hikojima 1"-"Tsuda" high as the strains "Giran" and "Ogawa"-"Kubo" group.

Thus the first five strains "Inaba"-"Suga" have such agglutinability as main type, "Hamburg", the final three strains "Ogawa"-"Kubo" reveal the same agglutinability as varied type, "Giran" and the four strains "Hikojima 1"-"Tsuda" behaved in agglutination, test once as the main type, and in other instances as the varied type.

Table I.

Cross agglutination test.

Strain \ Serum	Highest dilution showing positive reaction					
	Inaba	Hikoshima	Tsuda	Ogawa	Hamburg	Giran
Inaba	25600	12800	3200	800	3200	400
Sano	25600	12800	3200	800	3200	200
Wada	51200	12800	1600	800	3200	200
Chokichi	25600	12800	3200	800	3200	400
Suga	25600	12800	3200	800	3200	400
Hikojima 1	25600	12800	3200	1600	3200	1600
,, 2	25600	12800	3200	1600	3200	1600
,, 3	25600	12800	3200	3200	3200	800
Tsuda	25600	12800	3200	3200	3200	1600
Ogawa	25600	6400	1600	6400	200	1600
Hirahara	25600	6400	3200	6400	200	1600
Kubo	25600	6400	3200	6400	400	1600
Hamburg	21200	12800	3200	200	6400	400
Giran	25600	6400	1600	3200	800	1600
Suga H	25600	12800	3200	800	3200	200
,, T	25600	12800	3200	800	3200	200
,, W	sp	sp	sp	sp	sp	sp

H : transparent colony. T : turbid colony. W : dry dark colony.
sp : spontaneous agglutination.

The sera "Hamburg" and "Giran" are able to show the differences in the types of strains, and the sera "Hikojima" and "Ogawa" reveal the tendency to do so, but the sera "Inaba" and "Tsuda" agglutinate all strains equally high. This character of the sera seems to be due to the individuality of the animal used for immunisation, for sera made by the same strain vary in this ability. Kabeshima[5] and Oda[6] minded it should be due to the antigenic construction of the strains.

Agglutinin absorption test using the strains "Inaba", "Hikojima 1", "Tsuda" "Ogawa", "Hamburg", "Giran" and their corresponding sera, indicates that strain "Inaba" corresponds entirely to the main type and the strain "Ogawa" to the varied type, but the straims "Hikojima 1" and "Tsuda" differ in the antigenic constitution from the main type or the varied type, and they may thus be arranged as a third type.

Conclusion.

1) 12 strains of the cholera vibrio collected in Japan in 1921, reveal nothing new in microscopical, cultural and biological features.

2) Serologically, the strains from the summer epidemic correspond to the main type of Kabeshima, and those from the autumnal epidemic belong to the varied type of the same author. And the strains imported from Shanghai form possibly a third type.

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A simplified Method of finding Tubercle bacilli in Sputum, (especially the staining and collecting Method).

By

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(From the second Section of the Bacterio-Serologica Department.
Head of the Section. Colonel Kojo Saisawa.)

It is very important for the preventive and diagnostic measurement of respiratory tuberculosis that we should be able to find out easily tubercle bacilli in the sputum. For this purpose, devicing a simplified and excellent staining, and collecting method is an urgent necessity.

1. Staining method.

From the reports of many investigation and any experiments I should say that Konrich's method is most excellent out of various staining methods that are used now. Next noted to it is Bender's method which is a new method of picric acid stainings.

I have deviced a modified method by which I could eliminate the defects in Konrich's method arising from changeableness of decolorising material and too deep secondary counter staining, and accomplish decolorising and secondary counter staining at the same time. From my experiments, I declare that in point of positivity of baciili it is by no means behind to the original method and is specially fitted for smear specimen.

The method is as follows:

Konrich's method	Modified method
1. Staining under heating 2 minutes with Ziehl's carbolic fuchsin solution.	1. Same as original
2. Through by washing in water.	2. Same as original
3. Staining 1 to 2 minutes with 10% of sodium sulphide.	3. Treating for ½ to 1 minute with the mixture of 1 portion of M. A. C. or marachit green satulated solution and 2 portion of acetic acid which contains 30% pure acetic acid.

4. Washing with water	4. omitted
5. Secondary counter staining for 15 to 30 seconds with the mixture of 1 portion of marachit green satulated solution and 2 portions of water.	5. omitted
6. Washing with water	6. Same as original.

In addition to the modified Konrich's method I shall apply modified Bender's method, for the following reasons.

(a) As the material used in the modified Konrich's method has too strong acidity for staining specimens with collected bacilli, it is apt to decolorise already stained bacilli.

(b) Konrich's and modified Konrich's method are not fitted for color blindness.

(c) It is said that sometimes acid proof bacillus which is decolorised by alcohol is found in sputum. Therefore, nonalcoholic staining, so far as staining is concerned, may mislead us to take this kind of bacillus as tubercle bacillus.

I have used a simplified Bender's method mainly for staining specimens with collected bacilli, and found that it is by no means superior to the original method, and moreover, when the stained bacilli are treated with C A P solution, we can control the decolorization under inspection as this solution in light yellowish in color. Hence this method is most appropriate for staining specimens with collected bacilli which has weak resistance to decolorization.

Bender's method	Modified method.
1. Heating for 2 minutes with Ziehl's carbolic fuchsin solution.	1. Same as original
2. Washing with water.	2. Same as original
3. Decolorization with 3% hydrochloric acid alcohol.	3. Treatment for ½ to 1 minute with C A P solution (mixture of 3% hydrochloric acid alcohol and satulated picric solution in equal proportion.
4. Washing with water.	4. omitted
5. Light yellowish secondary staining with satulated picric acid solution.	5. omitted
6. Washing with water.	6. Same as original.

2. Collecting method.

After the comparison and trial of various collecting methods that have been reported, I found that compared to the method with antiformin, which has been most widely used for homogenizing sputum the method, with natrium hypochlorit

solution reported by Hermann Lorenz, has the adventage that its preparation is much easier and its expense is much cheaper, and homogenization is quicker and that the material treated with this solution has better adhesion on object glass. This fluid is prepared in the following way. Dissolve 286 gr. of sodium carbonate in 2l. of water. Add to it 215 gr. of chlor calcium (chlor content must be more than 25%; experimentally, industrial drift powder which contains much chlor will be convenient to use). After stiring up the mixture for a little while, let it stand still. Its supernatant fluid is taken and filtrated. It is kept in a cool and dark place from which it is taken out in a small bottle according to the need. As the effect of this solution entirely depends upon chlor content, it ought to be at least 2%, so that when this solution added to a strong mucous sputum and stired up well the latter will entirely be homogenised in 15–20 minutes.

The modification of precipitation method has aimed at to collect bacilli by utilizing the dissolving power of the drug and its specific gravity, without using a special apparatus I deviced a plan which could do centrifuge and yet superior to the original method. It is calcium precipitation method in which the velosity of precipitation, clearly noticeable white precipitate are well suited to the purpose of collecting bacilli.

The method is as follows:

(a) A very small portion of sputum to be examined is put aside.

(b) The remaining large portion of sputum is put into test tube of different sizes according to the need, and equal amount of Lorenz's natrium hypochlorit solution is added to it and the mixture is stired up. In 15 to 20 minutes already the macroscopically recognizable solid substances disappear and all are liquified.

(c) The liquid is made to stand still until air bubbles produced on the upper part all disappear.

(d) 10% chlor calcium solution is added to it in the same amount as Lorenz's solution; so that white turbidity is seen on it.

(e) Let it stand still for 10–20 minutes so that the volume of precipitate is reduced to minimum.

(f) The supernatant fluid is removed with pipet, and the precipitate is placed on many folded filter paper by which the water is absorbed (not filtrated) and the volume of the precipitate becomes still smaller and adheres slime like on the filter paper.

(g) The aboved dercribed slime like substance is mixed with a portion of sputum which was put aside and the mixture becomes semislime like substance. This is smeared on the object glass and dried in the air. When this is subjected fixing staining it becomes a beautiful specimen with collected bacilli.

The specimen made according to this method is almost always much better than that which is made with centrifuge. Most interesting is the fact that in the

specimen thus made, unlike the specimen made with centrifuge thers is not much difference of density according to the microscopic field and the distribution of bacilli in any part of the field of vision is equal and uniform.

Therefore this method should be conveniently employed when large amount of sputum is to be used and a large number of men are to be engaged in the work.

The Theory of the Wassermann Reaction with special Refference to the analogous specific Reactions between Heterophile (Forssman) Antibody and Tissue Lipoids.

By

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I. Review and discussion of the theory of the Wassermann reaction.

Gradual evolution of the antigen for the Wassermann reaction has resulted in separating decidedly the principle of the reaction from classical explanation of the well known phenomenon of complement fixation of the Bordet-Gengou reaction due to specific syphilitic antigens and antibodies. It was originally discovered by Wassermann, Neisser and Bruck[74] that aequeous extracts of congenital syphilitic liver possessed antigenic properties. Detre found independently and almost simultaneously a similar property in the extracts prepared from condylomata and other syphilitic tissues. Therefore, the antigenic power in the Wassermann reaction of such syphilitic tissue extracts was considered due simply to the existence of syphilitic antigen in the tissues.

It was soon found by Marie and Levaditi[36], however, that an aequeous extract of non-syphilitic liver had similar, though weaker, action to tissues containing syphilitic virus.

A remarkable revelation soon followed through the studies of Landsteiner, Müller and Pötzl[31] that alcoholic extracts of guinea-pig heart yielded results equal to those obtained withe an aequeous extract of syphilitic liver. Levaditi and Yamanouchi[31], Porges and Meier[50] also discovered, almost simultaneously, that the antigenic subtance could be extracted by means of alcohol from non-syphilitic human liver as well as from the syphilitic. Succeeding investigations of numerous workers confirmed and extended the original observation of Landsteiner and others, and it has been demonstrated that it is possible to substitute in the Wassermann reaction, for extracts of tissues containing syphilitic virus, the most varied sorts of tissue extracts, coming from tissues certainly free from syphilitic antigens; and that the antigenic properties reside to a considerable extent in the ether-soluble,

aceton-insoluble fraction of such alcoholic extracts (Noguchi[44]), even granting, as Mc. Lean[38] states, that the antigen is not "Lecithin", but something which is precipitated along with it. Again, the actual substance of pure culture of Spirohaete Pallida did not as a rule act as antigen with syphilitic sera in the reaction (Noguchi[45]). Hence it appears that the high efficiency of certain congenital syphilitic livers depends on autolysis rather than on the presence of abundant spirohaetes; although it is believed by some investigators (see Wells[79]) that when syphilitic liver extracts are used as antigen, we have a true Bordet-Gengou reaction of complement fixation with syphilitic substances present in this extract, in addition to the reaction which is accomplished by lipoids. On the other hand, the positive Wassermann reaction is said to have occured in Frambesia, Malaria, Leprosy, some kinds of febrile disease and others.

Thus, neither antigen nor reagin of the Wassermann reaction possesses any appreciable direct relationship with syphilitic virus. Since, it was established that the Wassermann reaction is not simply an example of the Bordet-Gengou phenomenon, a number of hypotheses have been advanced to explain the principle of the phenomenon.

Autoantibody formation: Weil and Braun[77,78] consider that in the course of disease, tissue products, such as albumin-lipoid complexes, are absorbed and give rise to antibodies. The antibody thus formed is supposed to have the power of reacting *in vitro* with the lipoidal component of the albumin-lipoid complex, the lipoid portion, of course, being alone present in alcoholic tissue extracts. Toyosumi's[70] observation that organ cells absorb the reacting bodies from the serum, has been cited in support of this theory (see also Guth, Breinl).

Toxolipoid-antibody: According to Citron's view[8], there is a syphilitic toxin which has an affinity for lipoids: the toxolipoid compound then acts as antigen and leads to the production of an antibody in the serum, which has the power of reacting *in vitro* with the lipoid by itself. The lipoid would thus stand in the same relationship to toxolipoid as diphtheria toxoid does to diphtheria toxin.

Weil and Braun's and Citron's theories rest on a similar basis, namely, the assumption that some body belonging to the class of antigens (tissue protein, syphilitic toxin) combined with lipoids give rise to an antibody. Thus the Wassermann reaction would be a kind of antibodyreaction (or immunity reaction) and complement would be fixed by an analogous mechanism to that which operates in the presence of specific antigen and antibody. However, a number of investigators have given up the view that auto-antibodies are concerned in the Wassermann reaction, as there was little experimental evidence in favour of this hypothesis. Thus, Levaditi and Yamanouchi[34], Periz[61], Friedemann[17], Manwarning[37], Kiss[28], Bruck[6,7], Epstein and Paul[11,12], Baumgärtel and others have offered a number of various hypotheses to explain the mechanism to inactivate complement in the Wassermann reaction.

Sachs[53, 54, 57] holds the view that the special behaviour of syphilitic sera to deprive complement of its haemolytic action in the Wassermann reaction should be attributed to the characteristic alteration of globulin of syphilitic serum where by the complement on digestion with organ extract is inactivated by such altered globulin, In support of the view, he cites the phenomenon which he observed with Altmann[55] and others, that conditions to inactivate complement in salt-free media acted similarly in the Wassermann reaction. Sachs's view is more or less in harmony with those of Friedemann, Schmidi[59], Liebens[35], Hirshfeld and Klinger[24], Bruck, Nathan[46, 47], Epstein and Paul and others. And since it was shown by the improved method of Sachs and Georgi[56, 57], Meinicke[40, 41] and others that the precipitum formation of syphilitic sera along with the Wassermann antigen ran parallel with Wassermann reaction in wide limits, and since it was agreed by many investigators (Sachs and his associates, Meinicke, Konitzer[29] and others) that the two reactions rest on the same principle, Sachs and his associates believe that the globulin of syphilitic sera has been altered in disperity (höhere Fällbarkeit) thus the adequate factors such as organ extracts lead to precipitate the syphilitic serumglobulin more readily and thus the inactivation of complement would be caused. This view appears to be most popular among recent investigators. Thus Wells[79] sums up the present state of the theory of the Wossermann reaction in this fashion: *A favorite interpretation of the Wassermann reaction, which seems to harmonize with the known facts, is that there is a precipitation of serum globulin by the lipoidal colloids of antigen and absorption of the complement by this precipitate.* Therefore, we shall discuss this hypothesis in detail before we come to our view. (1). Numerous workers have agreed that the reacting power of a syphilitic serum is resident in the globulin fraction (Landsteiner and Mueller[32], Gross and Volk[21], Noguchi[44], Bauer and Hirsch[2]).* This is also true of cerebrospinal fluids. (2). Apparently the globulin of syphilitic sera are altered in some way. Thus Noguchi states that in untreated or slightly treated case of primary and secondary syphilis, the globulin content of the serum is increased. According to Klausner[30], when distilled water is added in certain proportion to fresh serum, a distinct flocculent precipitate separates out in a few hours, and this property is much more marked in syphilitic than in normal sera. Bruck[7] states that the globulin of the syphilitic serum can be precipitated more readily and assumes a characteristic gelatinous appearrences; a simillar phenomenon has also been observed by adding ammonium sulphate, (Mac Donagh[39]). V. Dungern[11] states that heat coagulation of syphilitic serum is prevented by a smaller relative amount of an alkaline solution of indigo than in the case of normal sera and the reinvestigation of this phenomenon by Flood[19] and by Fujimoto[15] attributes the active constituents of the reagent simply to the alkalinity to the coagulation of

* According to the recent work of Taoka, the Wassermann reagin is not always precipitated along with globulin of the sera, but they will separate out from time to time either with albumine or globuline fraction depending on the method employed to fractionate the protein.

globulin. Syphilitic serum is said to floccurate on addition of appropriate colloidal suspensions which will not coagulate normal serum (Vernes[73]),

None of these properties of syphilitic sera, however, is at all specific for syphilitic infection, nor do they run paralell with the Wassermann reaction. Hence, these quantitative or qualitative differences of some syphilitic serum-globulin should not be readily admitted to have any direct relationship with the Wassermann reaction. (3). Hirschfeld and Klinger[24] believe that they have succeeded in converting active human sera from negative to positive Wassermann reaction by such treatment as to alter the physical condition of the globulin. Thus by shaking the sera with distilled water, or by digesting with organic or anorganic emulsion of agar or starch, specific serum-antiserum precipitate, barium sulphate, bacteria or blood corpuscle emulsion, the previously Wassermann negative sera give rise to positive reaction. Reinvestigating this phenomenon, Nathan, however, states that the property of such treated sera to inactivate complement along with tissue extract is not true positive Wassermann reaction, as the active power of such sera is thermolabile (heating half an hour at 55°C.) except in a few instances; and such sera will again turn to Wassermann negative by standing for a while; and again they never give rise to positive Sachs-Georgi reaction. He also cites the phenomenon, for the favour of his view, which was first observed by Sachs and Altmann[55] that the active non-syphilitic human sera sometimes give rise to false positive Wassermann reaction. Thus it is believed by majority of workers that such action of thermolabile constituents of some sera has nothing to do with the real constituents of the syphilitic serum to manifest positive Wassermann reaction. Hence, the observation of Hirschfeld and Klinger cannot afford any support to their globulin theory. (4). The author[66] is agreed with the view that the precipitum formation of syphilitic sera along with pure or mixed lipoidal emulsions (Porges and Meier[59], Elias, Neubauer, Porges and Salomon[38], Hermann and Perutz[28], Teruuchi and Toyoda[69], Meinicke, Sachs and Georgi and others) stands on the same principle with the Wassermann reaction; but regarding the part played by lipoid emulsion in the reaction, the author holds different views from Sachs and his associates and from Meinicke. Sachs's view is based on the experiment of Georgi[32] who saw that the precipitate obtained by digesting syphilitic sera along with lipoid emulsion was not completely redissolved in alcohol or in ether. Thus Sachs concludes that the precipitate mainly consists of globulin of the syphilitic serum, although the presence of lipoid from the emulsion is not excluded. But this experiment is not powerful enough to draw any decided conclusion from. Meinicke at first considered that the saltstable precipitate which was formed by his syphilis reaction consisted mainly of lipoids from the emulsion; but he gave up his former view and now believes that the precipitate chiefly consists of globulin of the syphilitic sera, since he has observed when the precipitation reaction is carried out by using lipoid emulsion which is stained with Sudan IV, the precipitate is practically colourless where the fluid remains

unchanged in colour. The author[66] observed, however, the precipitate in the Sachs-Georgi syphilis reaction contains practically the total amount of lipoids of the emulsion. Thus when the precipitation is completed as perfectly as possible by adding a suitable syphilitic serum until the supernatant fluid appears quite clear, it is found that an alcoholic extract obtained by treating the precipitate with an amount of alcohol equivalent to that originally present, is practically as efficacious as the original extract for producing haemolysis with Cobravenom; whereas the clear fluid, obtained after centrifuging off the precipitate, does not exhibit this property, except to a minimal degree. The author's view has been agreed with by many investigators that the precipitate obtained by the digestion of lipoid emulsion with syphilitic sera mainly consists of lipoids (Joel[27], Sheer[62], Niederhoff[49], Epstein and Paul[16], Taoka[72] and others). (5). Taoka, Wassermann[76] and Citron[9] states that they have succeded in obtaining an active substance of the syphilitic sera free from protein.

Thus there is no direct support to the hypothesis of Sachs and others. The recent work of Sachs, Georgi and others, who have established decidedly that the precipitum formation of the syphilitic serum with lipoid emulsion rests on the same principle with the Wassermann reaction, has surely brought out great advance for the investigation of the nature of the Wassermann reaction; yet the mysterious phenomenon has not yet been completely explained.

II. Is the Wassermann reaction lipoidphile antibody reaction?

The recent work of the author[65, 67] and others on heterophile (Forssman) antigen and antibody demonstrates decidedly that lipoids (especially phosphatides) can act as receptors in certain kinds of immunity reactions. Hence, it appears that there has been brought out powerful evidence to revive the old theories of Weil and Braun and others that the Wassermann reaction is a kind of lipoidophile antibody reaction.

The heterophile antibody manifests following specific reactions with the lipoids of heteropile tissues:

(1) Fixation of heterophile antibody with lipoid emulsions of heterophile antigens (Iwai[26], Georgi[23], Taniguchi[65]).

(2) The antibody fixes complement in the presence of specific lipoids (Taniguchi[65]).

(3) Precipitum formation of the lipoid emulsion by the antibody (Sordelli and Pico[63], Sachs and Guth, Taniguchi[67] and others).

Thus it can easily be seen that there exists close analogy between the manifestation of syphilitic sera with tissue lipoids (complement fixation and precipitation) and those of the heterophile antibody with the specific lipoids. The effect of secondary conditions (turbidity of the emulsion, the addition of cholesterol, salt content of the medium, temperature etc.) to carry out such lipoidotrophic biological reactions has been shown to exhibit strict parallelism both for syphilis reactions and for heterophile antibody reactions (Taniguchi). The only differences

distinctly demonstrated in the two kinds of reactions are concerned with the lipoids derived from different sources; thus, in contrast to the latter reaction, in the Wassermann reaction, there is no distinct specificity for lipoids which is used for antigens.

The lipoidophile antibody reactions appear to follow the general rule which has been established in the anti-protein immune body reaction. Thus, in addition to the 3 reactions of the heterophile antibody with the specific tissue lipoids, already mentioned, the interaction of the heterophile antibody with the specific lipoids *in vivo* appears to develop anaphylaxis (heterophile anaphylaxis, Taniguchi[68]). But, regarding (1) the specificity of the antigen and (2) the production of the antibody, there are distinct apparent differences between the proteinophile and lipoidophile antibody reactions. Just these two peculiar evidences have led previous investigators to give up the view, that the Wassermann reaction is an antibody reaction.

(1) **The specificity of the lipoidophile antibody reaction:** The discovery of heterophile antibody by Forssman[20], who found that tissues of guinea pig, when injected into the rabbit, engender in the rabbit's serum a highly active, haemolytic immune body for sheep corpuscles, has brought a great alarm for the specificity of immunity reactions. The distribution of heterophile antigen is so striking that there is no obvious relationship to the *species specificity* or to the group reactions of related species in contrast to the general rule which has been established in the anti-protein immunity reactions. Thus the same receptor for the heterophile antibody have been demonstrated on so widely divergent animal species such as organs of guinea-pig, horse, dog, cat, mouse, fowl, tortoise and others; where organs of man, rabbit, ox, goose, frog etc. lack the property. On the other hand, among animals so closely related as the ox and the sheep, and the rat and mouse etc., the organs of one may possess while the other lack heterophile receptors. The apparent curious distribution of the same receptor for the heterophile antibody has, however, a rational explanation, since it has been proved that the specific receptor is lipoids (Phosphatides) but not protein. The recent observations leave little room for doubting that specificity of the antibody reaction does depend upon chemical composition, and that the differences in species as exhibited by their biological reactions depend upon distinct differences of their proteins (see Pick[52], and Wells[79]). Hence, it would possibly be assumed the universal distribution of the same phosphatides, which can act as antigens in the Wassermann reaction, in the organic world; as phosphatides are rather simple in the chemical composion. This was anticipated in the case of heterophile antigens.

(2) **Antigenic power of lipoids.** Bang and Forssman[1] were the first who stated that they had succeeded in producing haemolytic immune body from ether soluble constituents of red blood corpuscles, but the majority of workers would hesitate to believe that the lipoid can act as antigens in the true sence (see Landsteiner[47]). Bruck[6] also failed to produce the Wassermann body by injecting

lipoids such as lecithins into animals. On the other hand, it is one of the cardinal points in Ehrlich's *Side-chain theory* that the receptor which fixes an antibody and the antigen which causes its development in the animal body are the same molecule with different names. But there were no experimental evidences whereby to consider that tissue lipoids act as antigen for generating antibody for the Wassermann reaction *in vivo*. But concerning the heterophile antibody reaction, it is decidedly established that lipoids can act as receptors *in vitro*, although they are practically devoid of the capacity of generating the antibody *in vivo* (Taniguchi). It would seem, therefore, in lipoidophile reactions the power to react specifically with the antibody *in vitro* (receptor) and the capacity for developing the antibody *in vivo* (antigen or immunogen) are to a marked extent independent properties. It appears, therefore, that the antigenic character of producing such lipoidophile antibody depends on some lipoidprotein complex, whereas the combining affinity resides in the lipoids. The author[67] has demonstrated definitely that the injection of watery emulsions of guinea-pig's organ (kidney and liver) into ihe rabbit causes the developement of so-called Wassermann body which is characterised by the property of fixing complement along with the Wassermann antigen derived from non-heterophile antigenic tissues; and the antibody nature of such property of the serum has been highly suggested by the parallel increase and decrease in amount with the heterophile antibody which has been simultaneously elicited by the procedure in the same animal. Of course, it is not known whether the Wassermann reactive body of human and animal sera are identical, but it has been generally agreed that the two properties are closely allied to each other. Thus, the possibility to develop the Wassermann body by the injection of animal tissues has been demonstrated.

Now the chief difficulty to maintain the lipoidophile antibody theory of the Wassermann reaction appears to have been explained by the analogy of the similar reaction of heterophile antigen and the antibody. Whether the known facts concerning the properties of syphilitic serum or the active constituents of the Wassermann reaction contradict the theory or not, should also be considered in this place.

(1) The fact that the active power of syphilitic sera or of cerebrospinal fluid for the Wassermann reaction resides in the globulin fraction does not contradict, but support the view, as the majority of antibodies are known to be mainly contained in the globulin fraction.

(2) The active constituents is known to pass through Belkefeld fillter (Seligmann and Pinkus[61]) as a majority of antibodies do (see Muir and Browning). Recent work of Yoshinare demonstrats that the active body of the Sachs-Georgi reaction also passes the filter.

(3) Michaelis and Skvirsky[43] (confirmed by many investigators) have shown that in the Wassermann reaction only the so-called mid-piece of the complement is fixed. The similar fact has also been observed in known antibody reactions;

thus the mixture of serum and antiserum, tuberculin and anti-tuderculin absorb only the mid-piece af complement.

(4) The occasional quantitative or qualitative variation of globulin or the other constituents of the serum is also expected in the course of such disease as syphilis.

(5) Physico-chemical factors play a part in the lipoidophile syphilis reaction, such as turbidity of the emulsion, addition of cholesterol, etc.; do not offer any contradiction to the hypothesis.

It appears, therefore, that the lipoidophile antibody reaction theory of the Wassermann reaction has been decidedly established. Wassermann[75] and Citron now hold the similar view with the author but from different experimental evidence.

III. The development of the Wassermann body in human subject.

As has been already emphasized that the Wassermann body is closely analogous to the heterophile antibody in the mechanism to react with lipoids as well as in the development. The two antibodies are known to be demonstrable in the normal serum of a certain kinds of animals. The presence of heterophile antibody in human sera has also been proved (see Taniguchi). It is known that the positive and negative Wassermann reaction of the human subject are not qualitative but quantitative (see Browning and Kennaway[4]). Thus the development of the wasseramnn body should similarly be cansidered as that of natural antibodies.

Nothing is known as to the development of the natural antibody; however, there is evidence that they develop in the course of physiological or pathological tissue disintegration. Suzuki[61] has found that natural anti-pig haemolysin is not usually present in the sera of young albino rats, but that it appears frequently in older animals, more abundantly in females than in males. He has also observed that this haemolysin specially increased in pregnancy and decreased after perturiation; in certain diseases also, such as infections of the lung, the haemolysin has been found to increase. The positive Wassermann reaction in syphilis has been said to be dependent upon the activities of the causative virus. Thus, as has been stated by Weil and others, it would perhaps be expected that in the course of the syphilitic infection, the tissue products such as lipoid-protein complex, are absorbed and elicite the Wassermann body.

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Contributions on the Study of the Forssman Antigen and Antibody.

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Introduction.

The species specificity is at the same time one of the most important and one of the most striking of the phenomena of immunity. The "Side-Chain" theory of Ehrlich takes its origin from this; and the clinical application of immunity reactions is also based mainly upon the same phenomenon.

A surprising fact, however, has been shown by Forssman (1911),[12] who proved that the injection of organ emulsion of the guinea-pig (the Forssman antigen) into rabbits leads to the development of a heterophile immune body for the red blood corpuscles of sheep (the Forssman antibody).

It was not only accepted immediately by a number of investigators, but it also became more mysterious when it was found that there was a wide distribution of the same antigen throughout the animal world without any appreciable relation to the animal species. Organs of the guinea-pig, horse, dog, cat, mouse, fowl and tortoise (animals of the guinea-pig type, Bail and Margulies 1913)[3] act in this way, but red blood corpuscles from these species do not contain this antigen; while those of the sheep and goat are able to generate this antibody, although their organs are devoid of this property. Furthermore, sheep sperm, as well as the gills of carp, pike and tench have been found to act as described.

On the other hand, the organs of the rabbit, man, ox, pig, rat, goose, pigeon, eel, frog, tai fish, etc., (animals of the rabbit type, Bail and Margulies) do not possess this antigen. The Forssman antigen, however different its origin may be, has the same character from an immunological point of view (Rezeptorengemeinschaft, Orudschiew 1913)[30]; accordingly, the antibody generated from it is of the same nature.

Forssman believed, at first, that the Forssman antibody need not necessarily possess any teleological or etiological significance, and considered that this phenomenon was contrary to the principle of Ehrlich's theory. Orudchiew investigated this point further, and proved that the generation of the antibody was

certainly responsive to the corresponding antigen. Thus, in this case too, the antigen specificity has been definitely proved, although the species specificity or the group reaction could not be observed; therefore, the theory of Ehrlich that the specificity of immunity reactions may depend on the chemical constitution of the antigen ("constitution specificity"), was strongly supported.

Recent works of Taniguchi (1921)[38] and others (Iwai 1917,[26] Friedberger and Suto 1919,[23] Sordelli and Pico 1920,[37] Sachs and Guth 1920)[34] have proved by the specific reactions in vitro (such as complement fixation, specific absorption and precipitation) that the chemical constituent of the specific molecule of the Forssman antigen is distinct from that of most of the other antigens; the former being regarded as lipoid (Overton) or phospholipin (Maclean), while the latter are supposed to be protein. Thus, the question of the specificity of the Forssman antigen and antibody reaction has been clearly settled for the first time.

It has also been shown by Forssman (1912)[14] and Hintze, that antisera containing the Forssman antibody are powerfully toxic for guinea-pig by intravenous injection. This phenomenon has been generally recognized as closely similar to the primary antiserum anaphylaxis, which was first discovered by Friedberger and Hartoch (1909,[17] 1909),[25] and afterwards confirmed by many succeeding investigators (Friedberger and Castelli 1910,[16] Doerr and Moldovan 1910,[6] Biedl and Kraus 1910,[4] v. Dungern and Hirschfeld 1911,[10] Kraus and Müller 1911,[27] Friedberger and Mita 1911,[18] Doerr and Weinfurther 1912).[7] Doerr and Pick (1913),[8, 9] Sachs and Nathan (1913)[35] considered that the symptoms above mentioned were a kind of inverse anaphylaxis due to the interaction between the Forssman antibody, which was injected, and the corresponding antigen, which was contained in the host.

Although much experimental evidence has been offered in support of this theory, Friedberger (1913)[19] and his associates (Friedberger and Schiff 1913,[21] Friedberger and Goretti 1914)[22] hesitated to admit it for the reasons that (1) any known specific reaction, such as precipitation, haemolysis and complement fixation, could not be observed between the serum or the red blood corpuscles of the host and the antisera, as in the case of anaphylaxis in the ordinary sense: (2) the toxicity of the antisera for the peculiar animal was not always quantitatively parallel to their haemolytic power for sheep corpuscles.

The chief difficulties against assuming that the specific toxicity is anaphylactic in nature have been adequately explained by Taniguchi and others; viz., (1) the lipoids of the tissues of animals of the guinea-pig type, which can be subjected to the specific intoxication (heterophile anaphylaxis, Taniguchi), are capable of demonstrating specific reactions such as complement fixation, precipitation and specific absorption; although the proteins lack this property. Thus an analogy with the reaction of the antigen and antibody in protein anaphylaxis seems to be lacking. (2) According to Taniguchi, heated antisheep haemolytic immune body can be classified into three types, viz., (a) serum from rabbits v. the Forssman

antigen contains merely lipoidophile antisheep haemolytic immune body (the Forssman antibody-toxic); (b) serum from guinea-pigs v. sheep corpuscles contains only pure proteinophile (isophile) antisheep haemolytic immune body (not toxic); (c) serum from rabbits v. sheep corpucles contains both a and b, that is a mixed type. Furthermore he has confirmed the fact that the toxicity of antisheep haemolytic immune sera from rabbits is due only to the Forssman antibody, and the isophile moiety lacks this property; thus the specific toxicity is almost strictly parallel to their contents in the Forssman antibody, but not to their haemolytic power.

Hence, it seems clear that the specific toxicity of the antisera containing the Forssman antibody is a kind of anaphylaxis (the Forssman antibody anaphylaxis) caused by the interaction between the Forssman antigen and antibody in vivo.

As human tissues do not contain the Forssman antigen, the conditions for anaphylactic intoxication are not provided, when the Forssman antibody is injected into the human subject. But the question arises as to whether heterophile anaphylactic reactions may also develop as the result of injecting the Forssman antigen into the human body, where naturally a variable amount of the Forssman antibody is present (see Friedemann 1917,[24] Taniguchi).

Amako (1912, 1914)[1,2] succeeded in producing an acute shock by injecting sheep or goat corpuscles (heterophile corpuscles) into rabbits which had been previously injected with the Forssman antigen from different animal species (organs of the guinea-pig, dog, tortoise and fowl) and had developed a large amount of the Forssman antibody in their sera; whereas, the injection of blood corpuscles from the ox, horse, dog, guinea-pig and tortoise had no effect. The injection of non-heterophile tissues (organs of the ox, pig, frog, etc.) did not render rabbits anaphylactic for heterophile blood corpuscles. He also observed, that the simultaneous injection of heterophile antisera with sheep or goat corpuscles into normal rabbits led to an acute shock. Therefore, it is suggested that a certain anaphylactic reaction may develop as the result of injecting the Forssman antigen into a human subject in virtue of the presence of the corresponding antibody.

In the present paper, an attempt has been made to ascertain whether pathogenic micro-organisms contain the Forssman antigen, or not, as that may throw light on the obscure phenomena of intoxication which are observable in consequence of injecting suspensions of certain kinds of bacteria, such as vaccine, into human subjects for curative or preventive purposes.

Distribution of the Forssman Antigen in Bacteria.

The Forssman antigen from animals, which has been found already, can be summarized as follows — (Forssman, Forssman and Widén 1911,[13] Amako, Forssman and Hintze, Doerr and Weinfurther, Morgenroth and Rosenthal 1912,[28] Bail and Margulies, Doerr and Pick, Friedberger, Friedberger and Poor 1914,[29] Friedberger and Schiff, Morgenroth and Bieling 1913,[29] Orudschiew, Pick 1913,[31] Sachs and

Nathan, Forssman and Fex 1914,[15] Friedberger and Goretti, Sachs and Georgi 1914,[52] Schiff 1914,[36] Tsuneoka 1914,[50] Weil 1914,[49] Friedemann, Iwai, Friedberger and Suto, Sordelli and Pico, Sachs and Guth, Taniguchi) ;

Guinea-pig : Kidney, adrenal, liver, heart, lung, brain, spleen, pancreas, large intestine, aorta, testicle, uterus, placenta, muscle, lens, serum, urine.

Horse : Kidney, adrenal, liver, heart, lung, brain, spleen, muscle, lens, serum, urine, lymph-gland.

Dog : Kidney, liver, heart, lung, brain, spleen, muscle, lens, serum, urine.

Cat : Kidney, liver, heart, lung, brain, spleen, muscle, lens, serum, urine.

Mouse : Kidney, liver, heart, lung, brain, muscle, cancer, sarcoma.

Fowl : Kidney, liver, heart, lung, brain, spleen, testicle, muscle, lens, serum, urine, egg-yolk.

Tortoise : Kidney, liver, heart, lung, brain, spleen, muscle, lens, serum, urine,

Sheep : Red blood corpuscles, sperm.

Goat : Red blood corpuscles.

Carp, pike, tench : Gill.

On the other hand, there has not been much research made with regard to the distribution of this antigen in micro-organisms. Forssman and Widén were the first who carried out experiments in connection with this subject. They reported that typhoid bacilli and brewers yeasts were not able to generate sheep haemolysin in rabbits, nor did the former react specifically with the Forssman antibody. Rothacker (1913)[32] found later whilst investigating the cause of meat poisoning, that antisera from rabbits, immunized with a mixture of paratyphoid B and Gärtner bacilli, showed a higher haemolytic power for the red blood corpuscles of sheep than sera from normal rabbits. Tsuneoka demonstrated that paratyphoid B, typhoid, coli, tubercle bacilli, staphylococci, vibrio Stade, and brewers yeasts were incapable of fixing the Forssman antibody in vitro

The above three investigations are the only ones that have been reported by the earlier investigators, concerning our present subject ; and none of them can be regarded as sufficient from which to draw any decided conclusion. Thus, in this work the following points have been examined to determine definitely whether the test materials contain the Forssman antigen or not.

1) The power of test materials to react specifically with known Forssman antibody in vitro ;
2) The capacity of test materials to generate a haemolytic immune body for goat red blood corpuscles by injecting into suitable animals ;
3) Whether the haemolytic immune body thus generated reacts specifically in vitro with known Forssman antigen or not.

As in the present paper I have attempted to show whether there exists a certain relationship between the toxicity of micro-organisms for the human subject

and the content in the Forssman antigen, or not, Shiga dysentery bacilli and cholera vibrio have been examined at first, from which the former is said to be powerfully toxic and the latter relatively less so. Typhoid, paratyphoid B and Gärtner bacilli have also been dealt with.

I. Methods.

Cultivation of Bacilli.

For the cultivation of bacilli however it must be borne in mind that broth made from horse meat is not suitable for this experiment, as it is known that horse meat contains a certain amount of the Forssman antigen. So I have used agar cultures, which were made from beef broth.

Animals for obtaining Antisera.

Rabbits of 1600 to 3620 grammes weight were used. Guinea-pigs are not suitable for the purpose, as it is well known that animals of the guinea-pig type are incapable of generating the Forssman antibody.

Immunization.

Bacilli were suspended in normal saline solution, the suspension was heated for one hour at 60°C., and injected in volume of 1.0 c.c. into the marginal vein of the ear. After each injection a small amount of blood was taken from the ear vein at intervals of about one week. The active sera thus obtained were put into quill tubes, and stored in the refrigerator.

Examination of Haemolysis.

Sera. — The sera were heated for 30 minutes at 56°C. before being used.

Suspension of red Blood Corpuscles and Complement. — The red blood corpuscles of goat were used; 0.5 c.c. of a 3 per cent. normal saline suspensions of washed sediment of goat's blood were tested respectively with 0.05 c.c. of guinea-pig's complement (5–10 M.H.D. for 0.5 c.c. of a 3 per cent. suspensions of ox corpuscles sensitized with excess of the corresponding immune body). Fresh guinea-pig serum sometimes contains a small amount of normal immune body for goat corpuscles, but when using 0.05 c.c. of this serum for complement, there is, as a rule, practically no lytic action on the test amount of goat corpuscles; thus it is not necessary to use the treated complement. In the case of normal immune body for ox corpuscles contained in fresh guinea-pig serum, it is necessary to treat all sera before use.

Procedures. — It is generally known that normal rabbit sera contain a certain amount of normol immune body for goat red blood corpuscles, which is demonstrated by Forssman, and others to be of the same nature as the Forssman antibody. In addition, in testing haemolytic power, the resistance of test red blood corpuscles as well as the activity of complement shall be taken into account. In order to avoid possible fallacies, I have simultaneously inactivated all specimens of sera from the same rabbit, and examined them in the same condition. The

results are recorded as follows after incubation for one and a quarter hours at 37°C., the tubes being shaken every quarter of an hour.

C = complete.
jc = just complete.
ac = almost complete.
vm = very marked.
m = marked.
d = distinct.
tr = trace.
ftr = faint trace.
o = none.

Examination of Agglutination.

Suspension of Bacilli. — One milligramme of an 18 hours pure culture was suspended in 1.0 c.c. of normal saline solution.

Procedures. — To each of a series of the tubes containing 0.5 c.c. of gradually diluted antiserum, two drops of the bacilli suspension were added. After 2 hours (4 hours in the case of Shiga dysentery bacilli) incubation at 37°C., the tubes were allowed to stand in the ice-chest overnight, and then the results were recorded (###, ##, #, +, ±, —, respectively, indicate the intensity of the agglutination).

In this case too, the possibility of fallacies have been avoided.

II. Experiments with Shiga Dysentery Bacilli.

A. Specific Reactions of Shiga Dysentery Bacilli for the Forssman Antibody.

1. Fixation of the Forssman Antibody by Shiga Dysentery Bacilli.

Method A (Table I. A.)

Antisera. —

1) Serum of rabbit No. 17 (Forssman antibody, M.H.D. 0.0002 c.c., drawn off 11 days after the third injection of 10. c.c. of a 10 per cent. saline emulsion of guinea-pig kidney).

2) Serum of rabbit No. 16 (antiox haemolytic immune body, M.H.D. 0.001 c.c., drawn off 11 days after the third injection of washed ox red blood corpuscles).

Procedures. —

Thirty milligrammes of an 18 hours culture of Shiga dysentery bacilli were added to each of the tubes containing 1.0 c.c. of each antiserum diluted with saline solution (contains 125 M.H.D.). After one hour's digestion in the ice-chest the tubes were centrifuged. The haemolytic power of the antisera thus treated was then examined.

Method B (Table I. B.)

Antisera. —

1) Serum of rabbit No. 7 (Forssman antibody, M.H.D. 0.002 c.c., obtained by the same treatment as antiserum No. 17).

2) Serum of guinea-pig No. 5 (pure isophile antigoat haemolytic immune body, M.H.D. 0.005 c.c., drawn off 15 days after a single injection of washed goat red blood corpuscles).

Procedures. —

Thirty milligrammes of an 18 hours culture of Shiga dysentery bacilli were added to each of the tubes containing 1.0 c.c. of each antiserum diluted with saline solution (contains 100 M.H.D.). After one hour's incubation at 37°C., the tubes were centrifuged. A descending amount of the antisera thus treated was added to several tubes containing 0.5 c.c. of a 3 per cent. suspension of goat red blood corpuscles. After one hour's digestion in the ice-chest, the tubes were centrifuged, and the supernatant fluids were pipetted off. Finally the sediments of corpuscles were resuspended in 1.0 c.c. of saline solution, and the haemolysis test of the suspensions was carried out.

Table I.

A

Amount of antiserum in						
c.c.		0.3	0.1	0.05	0.03	0.01
M.H.D.		37.5	12.5	6.25	3.75	1.25
Serum of rabbit No. 17.	treated	d	tr	c	c	c
	not treated	c	c	c	c	c
Serum of rabbit No. 16.	treated	c	c	c	c	ac
	not treated	c	c	c	c	c

B

Amount of antiserum in						
c.c.		0.3	0.1	0.05	0.03	0.01
M.H.D.		30	10	5	3	1
Serum of rabbit No. 7.	treated	c	m	tr	c	c
	not treated	c	c	c	c	c
Serum of guinea-pig No. 5.	treated	c	c	c	c	ac
	not treated	c	c	c	c	c

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, and complement = 0.

As illustrated in Table I. A. B., the haemolytic power of the Forssman antibody (sera of rabbits Nos. 17 and 7) treated with the Shiga dysentery bacilli was much weakened compared with that of its control; while this was not the case with the sera of rabbit No. 16 and guinea-pig No. 5. But it is to be noted

that, according to the Colloid-chemical reaction, the immune body will be non-specific adsorbed by a substance with such a very small surface as bacteria. However, in the case with sera of rabbit No. 16 and guinea-pig No. 5 we cannot find any trace of the adsorption. Hence, there is a strong reason to consider that the fixation of the Forssman antibody by the Shiga dysentery bacilli is due to their specific absorption. An important fact, however, which seems to attract our attention, is that the result, which the antiserum of rabbit No. 17 suffered in the method A, was greater than that which the antiserum of rabbit No. 7 suffered in the methods B, both being the Forssman antibody. It may be expected that in the first experiment some combinations of the antiserum and the bacilli extracts will fix the complement to a certain extent, but in the second such an effect as due to the complement fixation will be absolutely excluded. Thus, the inhibition of the haemolysis in the former might be the sum of the effect due to the specific absorption by the bacilli themselves and that due to the complement fixation, while in the latter only the effect due to the specific absorption was the case. But it appears to us that, as regards the specificity a strong support was given by this complement fixation, which seems to have happened at the same time in the method A along with the specific fixation, because in the case with the antiserum of rabbit No. 16 (antiox haemolytic immune body) no trace of complement fixation was found.

2. Complement Fixation exhibited by the Forssman Antibody in the Presence of watery Extracts of Shiga Dysentery Bacilli.

Methods.

Antiserum.

Serum of rabbit No. 17 (Forssman antibody, above mentioned).

Antigen.

Four milligrammes of a 24 hours culture of Shiga dysentery bacilli were suspended in 1.0 c.c. of normal saline solution; the suspension was heated for one hour at 60°C., and allowed to stand in room temperature overnight, and the supernatant fluid was employed as antigen after centrifuging thoroughly.

Procedures.

To each of a series of the tubes containing 0.5 c.c. of the antigen 0.05 c.c. of native as well as antiserum treated with goat red blood corpuscles was added along with ascending doses of complement. The mixture was incubated for one and a half hours at 37°C., the tubes being shaken every quarter of an hour. Then 0.5 c.c. of a 5 per cent. suspension of ox corpuscles sensitized with excess of the corresponding immune body (with 7.5 M.H.D.) was added to each tube. The results were recorded after a further incubation for one and a quarter hours at 37°C. (Table II).

Table II.

Antiserum 0.05 c.c.	Doses of Complement					
	4	6	8	10	12	14
Native	o	o	o	m	vm	c
Treated	m	ac	c	c	c	c

M.H.D. of complement. 0.0075 c.c.

Control. — Serum alone with 4 doses of complement in saline solution = c.
Antigen alone with 4 doses of complement = c.
Complement control = 0.0075 c.c.

In this complement fixation test the difference in the amount of complement in successive tubes is 2 M.H.D. (4, 6, 8, 10, etc.), and the amount of complement contained in the tube immediately preceding that which gives complete haemolysis, is taken as the amount of complement which is fixed. As illustrated in Table II., the Forssman antibody has fixed 12 doses of the complement in the presence of the watery extracts of Shiga dysentery bacilli, while the treated antiserum, which was therefore deprived of its Forssman antibody, has fixed only 5 doses of the complement. It has been shown by Taniguchi that the Wassermann reacting body develops parallel to the Forssman antibody in rabbits. I have examined the contents of the Wassermann reacting body contained in the serum of rabbit No. 17 (the Forssman antibody) according to the method of Browing (1919),[5] and have proved that 5 doses of complement have been fixed too. So the 5 doses of the complement, which are fixed by the treated antiserum in this experiment are due mainly to the Wassermann reacting body. Then the difference in the amount of the complement (7 doses) in both series of the test must be accounted for by the Forssman antibody, which is contained in the native antiserum.

The results in this section are summarised as follows:

1) Shiga dysentery bacilli fix the Forssman antibody specifically in vitro.
2) Watery extracts of the bacilli are capable of fixing complement in vitro along with the Forssman antibody.

B. Are Shiga Dysentery Bacilli Capable of Generating the Forssman Antibody in Vivo?

1. Quantitative Examination of Haemolysin for Goat red Blood Corpuscles.

Twelve rabbits varying in weight from 2400 to 3500 grammes were used. Various amounts of the bacilli (0.0125–0.5 milligramme) were injected from 1 to 5 times. A small quantity of blood was drawn off several times after intervals of from 3 to 10 days.

The contents of haemolysin for goat red blood corpuscles as well as of specific agglutinin were tested (Table III, (1). (2).).

Table III (1).

Number of rabbit	Serum obtained after	Weight of rabbit (in gramme)	Amount of bacilli (in milligramme)	Interval of bleeding (in days)	Minimum haemolytic dose (c.c.)	Titer of agglutinin (dilution of antiserum)
No. 18	(Normal)	2900	—	—	0.2 (m)	40
	I. inj.	2820	0.025	3	0.05	80
	II. „	2280	0.050	5	0.03	5120
	III. „	2870	0.120	6	0.008	5120
	IV. „	2700	0.250	8	0.008	5120
	V. „	2600	0.500	7	0.008	5120
No. 19	(Normal)	2600	—	—	0.2 (ac)	20
	I. inj.	2660	0.025	3	0.05	320
	II. „	2700	0.050	5	0.005	10240
	III. „	2760	0.120	6	0.02	10240
	IV. „	2670	0.250	8	0.009	5120
	V. „	2520	0.500	7	0.05	5120
No. 20	(Normal)	2400	—	—	0.05	160
	I. inj.	2370	0.025	3	0.04	160
	II. „	2420	0.050	5	0.004	10240
No. 21	(Normal)	2720	—	—	0.05	320
	I. „	2670	0.025	3	0.02	320
	II. „	2630	0.050	5	0.009	5120
No. 22	(Normal)	2500	—	—	0.04	40
	I. „	2600	0.025	3	0.02	320
No. 23	(Normal)	3500	—	—	0.06	20
	I. „	3480	0.025	3	0.06	5120
	II. „	3300	0.050	5	0.05	640
	III. „	3150	0.100	7	0.02	5120
	IV. „	3140	0.200	6	0.02	2560
No. 25	(Normal)	3500	—	—	0.02	40
	I. inj.	3250	0.0125	3	0.02	160
	II. „	2660	0.0250	6	0.03	2560
	III. „	2510	0.0500	5	0.02	2560
	IV. „	2450	0.1000	5	0.05	2560

Table III (2).

Number of rabbit	Serum obtained after	Weight of rabbit (in gramme)	Amount of bacilli (in milli gramme)	Interval of bleeding (in days)	Minimum haemolytic dose (c.c.)	Titer of agglutinin (dilution of antiserum)
No. 26	(Normal)	2770	—	—	0.1	20
	I. inj.	2500	0.0125	3	0.04	160
	II. „	2280	0.0250	6	0.02	5120
	III. „	2130	0.0500	5	0.02	5120
	IV. „	1930	0.1000	5	0.01	5120
No. 27	(Normal)	2980	—	—	0.02	80
	I. inj.	2830	0.0125	3	0.02	80
	II. „	2400	0.0250	6	0.006	2560
	III. „	2370	0.0500	5	0.007	5120
	IV. „	2150	0.1000	5	0.007	2560
No. 28	(Normal)	2830	—	—	0.2 (ac)	40
	I. inj.	2720	0.0125	3	0.06	80
	II. „	2630	0.0250	6	0.06	640
	III. „	2640	0.0500	5	0.03	1280
	IV. „	2460	0.1000	5	0.007	2560
No. 30	(Normal)	3480	—	—	0.2 (Vm)	40
	I. inj.	3050	0.025	4	0.09	640
	II. „	2910	0.050	6	0.04	1280
	III. „	2790	0.100	6	0.005	1280
	IV „	2900	0.200	6	0.003	2560
No. 31	(Normal)	3320	—	—	0.05	40
	I. inj	3200	0.025	4	0.01	320
	II. „	2680	0.050	6	0.002	2560
	III. „	2520	0.100	6	0.004	1280

Controls for serum, corpuscles suspension, complement, and saline solution = 0 and —.

As will be seen in Table III (1), (2), haemolysin for goat red blood corpuscles has increased roughly parallel to the development of specific agglutinin for Shiga dysentery bacilli in every case except one (rabbit No. 25), though the amounts of the bacilli, which were injected, were very small (the maximum amount being 0.5 milligramme).

Now, the questions which naturally arise concerning the cause of the development of the haemolysin may be summarized as follows: —

1) Have the Shiga dysentery bacilli a specific antigen for the goat haemolysin?
2) Is it an effect due to a non-specific stimulus?
3) Is it a daily fluctuation?

The first and second questions are of etiological significance, which touch

on the reason of the development of the haemolysin, so they are dealt with later. In order to solve the third question, I have taken a small amount of blood from 8 normal rabbits 2 or 3 times at intervals of 5 and 12 days. All specimens from the same rabbit were heated at the same time, and their lytic action was tested under the same conditions simultaneously (Table IV.).

Table IV.

Number of rabbit	Weight of rabbit (in gramme)	Times of bleeding	Interval of bleeding (in days)	Haemolysis				
				Amount of antiserum (c.c.)				
				0.2	0.1	0.07	0.05	0.03
No. a	2000	I	—	c	ac	vm	m	d
	2050	II	5	c	vm	m	m	d
	1860	III	5	c	vm	m	m	d
No. b	2150	I	—	ac	vm	m	m	d
	2200	II	5	ac	vm	m	m	d
	2060	III	5	ac	vm	m	m	d
No. c	1950	I	—	vm	vm	m	m	d
	1750	II	5	vm	m	m	d	tr
	1930	III	5	vm	m	m	d	tr
No. d	2220	I	—	ac	vm	vm	m	d
	2040	II	5	ac	vm	m	m	d
No. e	2350	I	—	c	vm	vm	m	d
	2180	II	5	c	vm	vm	m	d
	2000	III	5	c	vm	vm	m	d
No. f	3550	I	—	c	c	c	c	c
	3200	II	12	c	c	c	c	ac
No. g	3220	I	—	c	c	c	ac	vm
	3120	II	12	c	c	c	vm	vm
No. h	2470	I	—	c	c	c	c	c
	2350	II	12	c	c	c	c	c

Controls for serum, corpuscles suspension and complement = 0.

As illustrated in Table IV, the lytic action of every specimen from the same rabbit on the test amount of goat red blood corpuscles has shown practically no daily fluctuation. Hence; it is clear that the increase of the goat haemolysin in the main test above mentioned is an effect due to the injection of Shiga dysentery bacilli into rabbits.

2. **Qualitative Examination of the Haemolysin for Goat red Blood Corpuscles.**

It is an important fact to determine, whether the haemolysin is a product

due to the specific antigen contained in Shiga dysentery bacilli, or not. Moreover, the relationship of the haemolysin to the specific agglutinin, and the presence of another haemolysin (group-and isohaemolysin) are examined.

1) Specific Fixation of the Haemolysin by Shiga Dysentery Bacilli.

Method A (Table V. A.).

Antiserum.

Serum of rabbit No. 28 (Shiga immune serum, M.H.D. 0.008 c.c., drawn off 10 days after the fourth injection of Shiga dysentery bacilli).

Procedures.

Thirty milligrammes of an 18 hours culture of Shiga dysentery bacilli were added to a tube containing 1.0 c.c. of the antiserum (contains 125 M.H.D.). The mixture was placed for one hour in the ice-chest, the tube being shaken several times. The supernatant serum, after centrifuging was then examined for its haemolytic and agglutinating power.

Method B (Table V. B.).

Antisera.

1) Serum of rabbit No. 30 (Shiga immune serum, M.H.D. 0.003 c.c., drawn off 8 days after the fourth injection of Shiga dysentery bacilli).

2) Serum of guinea-pig No. 5 (pure isophile antigoat haemolytic immune body, already mentioned).

Procedures.

Thirty milligrammes of an 18 hours culture of Shiga dysentery bacilli were added to each of the tubes containing 1.0 c.c. of antiserum diluted with saline solution (contains 100 M.H.D.). After one hour's incubation at 37°C., the tubes were centrifuged. A descending amount of the antisera thus treated was added to several tubes containing 0.5 c.c. of a 3 per cent. goat red blood corpuscle suspension. After one hour's digestion in the ice-chest, the tubes were centrifuged, and the supernatant fluid was pipetted off. The sediments of the corpuscles were then resuspended in 1.0 c.c. of saline solution, and the haemolysis test was carried out. The treated antiserum of rabbit No. 30 was examined for its specific agglutinating power.

Table V.

A

Antiserum	Serum of rabbit No. 28 (Shiga immune serum)								
Reaction	Haemolysis					Agglutination			
Amount of antiserum in c.c.	0.3	0.1	0.05	0.03	0.01				
Amount of antiserum in M.H.D.	37.5	12.5	6.25	3.75	1.25				
Dilution of antiserum						10	20	1280	2560

Treated	tr.	0	0	0	0	+	—	—	—
Not treated	c	c	c	c	c	++	++	++	+

B

Antiserum	Serum of rabbit No. 30 (Shiga immune serum)					Serum of guinea-pig No. 5 (pure isophile anti-goat haemolytic immune body)				
Reaction	Haemolysis									
Amount of antiserum in c.c.	0.3	0.1	0.05	0.03	0.01	0.3	0.1	0.05	0.03	0.01
Amount of antiserum in M.H.D.	30	10	5	3	1	30	10	5	3	1
Treated	ac	tr	0	0	0	c	c	c	c	ac
Not treated	c	c	c	c	c	c	c	c	c	c

Reaction	Agglutination				
Dilution of antiserum	10	20	50	1280	2560
Treated	+	—	—	—	—
Not treated	++	++	++	++	+

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, complement, and saline solution=0 and—.

As will be seen in Table V, A, and B, after contact with Shiga dysentery bacilli the antisera of rabbits Nos. 28 and 30 lost a great part of the haemolysin as well as of the agglutinin.

Specific agglutinin for Shiga dysentery bacilli is, of course, a specific product responsible to the injection of Shiga dysentery bacilli. Therefore it is a matter of course, that the specific agglutinin was specifically absorbed by the bacilli. On the other hand, it has been shown by these two experiments that the haemolysin contained in the Shiga immune sera was absorbed, like the specific agglutinin, by the bacilli. If the haemolysin is a product due to a non-specific stimulus induced by the bacilli, this fixation of the haemolysin will not occur. Furthermore the haemolysin must be thermostable, because heated sera were always tested; and when we look at the course of the haemolysin, we note a slow diminution after a certain period of time. These two features are the important characteristic of an immune body. Therefore, there is a strong reason for determining that the haemolysin for goat red blood corpuscles developed by injecting Shiga dysentery bacilli into rabbits is a product due to the specific antigen contained in the bacilli. Therefore, the haemolysin thus generated is a thermostable immune body, viz. an amboceptor.

2) Examination of Relationship between the Haemolysin for Goat Corpuscles and the specific Agglutinin.

Methods.

Antisera.

1) Serum of rabbit No. 18 (Shiga immune serum, M.H.D. 0.008 c.c., drawn off 8 days after the fifth injection of Shiga dysentery bacilli).

2) Serum of rabbit No. 28 (Shiga immune serum, already described).

Procedures.

A quantity of 1.0 c.c. of washed goat red blood corpuscles was added to each of the tubes containing 1.0 c.c. of antiserum (contains 125 M.H.D.). After one hour's digestion in the ice-chest the tubes were centrifuged, and the supernatant sera were retained. And with antisera thus treated the haemolysis and agglutination tests were carried out (Table VI).

Table VI.

Antiserum	Serum of rabbit No. 18 (Shiga immune serum)			Serum of rabbit No. 28 (Shiga immune serum)					
Reaction	Haemolysis								
Amount of antiserum in c.c. / M.H.D.	0.3 / 37.5	0.1 / 12.5	0.07 / 8.75	0.3 / 37.5	0.1 / 12.5	0.07 / 8.75			
Treated	0	0	0	0	0	0			
Not treated	c	c	c	c	c	c			
Reaction	Agglutination								
Dilution of antiserum	10	160	1280	5120	10240	10	160	1280	5120	10240
Treated	###	###	###	+	−	###	###	###	+	−
Not treated	###	###	###	+	−	###	###	###	+	−

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, complement, and saline solution=0 and—.

As will be seen in Table VI, after treatment with goat red blood corpuscles the Shiga immune serum was totally deprived of its goat haemolysin. But it agglutinated Shiga dysentery bacilli in the same degree as the native antiserum. It is proved by this experiment that the goat haemolysin and the specific agglutinin contained together in the Shiga immune serum are quite distinct from each other, and certainly of different mechanism.

3) Examination of Group-and Isohaemolysins.

As shown by Forssman, and Orudschiew, the haemolytic power of the Forssman antibody is specific only for sheep and goat red blood corpuscles, but

it does not contain such a grouphaemolysin as an immune body for ox corpuscles, which is accompanied, as a rule, by an immune body from rabbits v. sheep or goat corpuscles (the mixed type, Taniguchi), as well as from guinea-pigs v. sheep or goat corpuscles (the pure isophile, Taniguchi). As already demonstrated, Shiga immune serum contains, like the Forssman antibody, an immune body for sheep and goat corpuscles; but it is important to examine whether Shiga immune serum contains any kind of group-and isohaemolysin or not.

Methods.

Antisera.

1) Serum of rabbit No. 18 (Shiga immune serum, already described).
2) Serum of rabbit No. 7 (Forssman antibody, already described).

Red Blood Corpuscles.

The red blood corpuscles of the ox, man, guinea-pig, and rabbit were examined. When the red blood corpuscles of guinea-pigs were tested, 0.1 c.c. of fresh serum from normal rabbits was used for complement (Table VII).

Table VII.

Red blood corpuscles of	Serum of rabbit No. 18 (Shiga immune serum)					Serum of rabbit No. 7 (Forssman antibody)				
	Amount of antiserum (c.c.)					Amount of antiserum (c.c.)				
	0.3	0.1	0.01	0.008	0.007	0.3	0.1	0.01	0.002	0.001
Ox	0	0	0	0	0	0	0	0	0	0
Man	0	0	0	0	0	0	0	0	0	0
Guinea-pig	0	0	0	0	0	0	0	0	0	0
Rabbit	0	0	0	0	0	0	0	0	0	0
Goat	c	c	c	c	ac	c	c	c	c	ac

M.H.D. of complement. 0.0075 c.c.
Controls for serum, corpuscles suspension, and complement = 0.

As shown in Table VII, the Shiga immune serum contains, like the Forssman antibody, nothing of group-and isohaemolysins.

The results in this section are summarised as follows: —

1) Shiga dysentery bacilli are capable of generating goat haemolysin when injected into rabbits.
2) The haemolysin thus generated is an amboceptor responsible to the specific antigen contained in the bacilli, therefore it is absorbed by the bacilli specifically. But it is quite distinct from the agglutinin.
3) The haemolytic power of the haemolysin is only specific for goat and sheep red blood corpuscles, but there is an absence of any kind of group-and isohaemolysins.

C. Specific Reaction of Shiga immune Serum with known Forssman Antigen.

1. Specific Fixation of Shiga immune Serum by Cell Suspension

Antisera.

1) Serum of rabbit No. 28 (Shiga immune serum, already mentioned).

2) Serum of rabbit No. 30 (Shiga immune serum, already described).

3) Serum of rabbit No. 7 (Forssman antibody, already described).

4) Serum of guinea-pig No. 2 (pure isophile antigoat haemolytic immune body, M.H.D. 0.005 c.c., drawn off 15 days after a single injection of washed goat red blood corpuscles).

5) Serum of rabbit No. 16 (antiox haemolytic immune body, above described).

Preparation of Cell Suspensions.

A portion of tissue (free from fat) was minced and ground up well in a mortar, about 10 volumes of saline solution were added, and the mixture was filtered through gauze: then the suspension was repeatedly washed with saline solution in the centrifuge (7–8 times), till the supernatant fluid became perfectly clear; finally, one vulume of the sediment was resuspended in 4 volumes of saline solution. For the Forssman antigen the kindney of a normal guinea-pig was used, and for the non-Forssman antigen the kindney of a normal rabbit.

Procedures.

A quantity of 1.25 c.c. of each cell suspension was added to each of the tubes containing 4.0 c.c. of each antiserum diluted with saline solution (contains 500 M.H.D.). The mixtures were incubated for one hour at 37°C., the tubes being shaken several times. After centrifuging, the haemolytic doses of the supernatant fluid, were tested. In determining the haemolytic power of the treated antisera, the dilution due to the saline solution present in the cell suspension must, of course, be taken into account (Table VIII).

Table VIII.

Main Test

Cell Suspension of	Number of antiserum	Amount of antiserum (c.c. and M.H.D.)						
		1.0	0.5	0.3	0.1	0.05	0.03	0.01
		100	50	30	10	5	3	1
Guinea-pig Kidney	Serum of rabbit No. 28	0	0	0	0			
	„ No. 30	0	0	0	0			
	„ No. 7	0	0	0	0			
	Serum of guinea-pig No. 2				c	c	c	c
	Serum of rabbit No. 16				c	c	c	ac

Rabbit Kidney	Serum of rabbit No. 28				c		c		c		vm
	,, No. 30				c		c		c		c
	,, No. 7				c		c		c		vm
	Serum of guinea-pig No. 2				c		c		c		c
	Serum of rabbit No. 16				c		c		c		ac

Control (Not Treated)

Serum of rabbit No. 28	c	c	c	c	c	c		vm
,, No. 30	c	c	c	c	c	c		c
,, No. 7	c	c	c	c	c	c		vm
Serum of guinea-pig No. 2				c	c	c		c
Serum of rabbit No. 16				c	c	c		ac

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension and complement=0.

As will be seen in Table VIII, the goat haemolysin contained in the sera of rabbits Nos. 28 and 30 (Shiga immune sera) has been fixed in just the same manner as the Forssman antibody by the cell suspension of the guinea-pig kidney, which acts as the Forssman antigen. But the sera of guinea-pig No. 2 (pure isophile antigoat haemolytic immune body) and rabbit No. 16 (antiox haemolytic immune body) have not been effected by this treatment. On the other hand the suspension of rabbit kidney, which does not contain the Forssman antigen, was quite indifferent against all antisera. Moreover, I have found that the Shiga immune sera thus treated were similarly never deprived of their agglutinin, as has been already shown in their absorption experiment with goat red blood corpuscles.

2. Specific Fixation by a alcoholic Extracts

It has been shown by Iwai and Taniguchi independently that when the Forssman antibody is mixed with saline emulsions of alcoholic extracts derived from a tissue which acts as the Forssman antigen, the former is fixed by the latter to a varying degree. This reaction has become one of the important features of the Forssman antibody. Behaviour of the goat haemolysin contained in Shiga immune serum for this reagent was tested.

Methods.

Antisera.

1) Serum of rabbit No. 31 (Shiga immune serum, M.H.D. 0.002 c.c., taken 6 days after the second injection of Shiga dysentery bacilli).

2) Serum of rabbit No. 17 (Forssman antibody, above mentioned).

3) Serum of rabbit No. 16 (antiox. haemolytic immune body, already described).

Preparation of alcoholic Extracts.

One part of well minced tissue (free from fat) was added to 9 parts of 96 per cent. alcohol, and the mixture was allowed to stand at room temperature for about 7 days, being shaken up several times; the clear extract was then filtered through fat-free paper and kept in the dark in stoppered bottles. For the Forssman antigen the heart of a normal guinea-pig was used and for the non-Forssman antigen the heart of a normal ox was employed.

Procedures.

Three parts of each alcoholic extract were evaporated in the incubator to one part. One part of the extracts thus evaporated was added slowly respectively to 9 parts of normal saline solution, and saline emulsions of maximum turbidity were made. Varying M.H.D. of each antiserum were added to each of the tubes containing 0.5 c.c. of each saline emulsion. The mixtures were incubated for one hour at 37°C., the tubes being shaken several times. Then 0.5 c.c. of a 3 per cent. goat or ox red blood corpuscle suspension was added to each tube. After one hour's digestion in the ice-chest the tubes were centrifuged, and the supernatant fluids were pipetted off. Finally the sediments of corpuscles were resuspended in 1.0 c.c. of normal saline solution, and the haemolysis test was carried out (Table IX).

Table IX.

Alcoholic extracts of	Number of antiserum	Amount of antiserum (c.c. and M.H.D.)				
		0.2 / 20	0.15 / 15	0.1 / 10	0.05 / 5	0.03 / 3
Guinea-pig heart	Serum of rabbit No. 31	d	tr	0	0	0
	,, No. 17	tr	0	0	0	0
	,, No. 16	c	c	c	c	c
Ox heart	,, No. 31	c	c	c	c	c
	,, No. 17	c	c	c	c	c
	,, No. 16	c	c	c	c	c
Not treated	,, No. 31	c	c	c	c	c
	,, No. 17	c	c	c	c	c
	,, No. 16	c	c	c	c	c

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, and complement = 0.

As illustrated in Table IX, the goat haemolysin contained in the Shiga immune serum was fixed to a great extent, like the Forssman antibody, by the alcoholic extract derived from the guinea-pig's heart. But the alcoholic extract of the ox heart had no influence on either of the antisera. Likewise the serum of rabbit No. 16 (antiox haemolytic immune body) suffered no change by these two reagents.

3. Comlement Fixation exhibited by alcoholic Extracts.

Taniguchi has established the fact that the Forssman antibody has a capacity for fixing complement in the presence of alcoholic organ extracts. And the amount of the complement fixed by it is much greater with extracts of organs which act as the Forssman antigen than with others. This property of the Forssman antibody became one of the most characteristic and most useful features in immunity reactions. Therefore, I have examined Shiga immune serum with this reaction.

Methods (Table X).

Antisera.

1) Serum of rabbit No. 30 (Shiga immune serum, already described).
2) Serum of rabbit No. 7 (Forssman antibody, already described).

Antigen.

Two parts of each crude alcohol extract, described in the preceding item, were evaporated in the incubator down to one part. One part of the extract thus condensed was slowly added respectively to 9 parts of normal saline solution, and saline emulsions of maximum turbidity were used as antigen.

Procedures.

0.05 c.c. of each antiserum was added to each of the tubes containing 0.5 c.c. of each antigen along with ascending doses of complement. The further procedures were quite similar to those mentioned in the previous complement fixation test.

Table X.

Antigen derived from	Number of antiserum	Doses of Complement					
		3	6	9	12	15	18
Guinea-pig heart	Serum of rabbit No. 30	0	0	0	0	0	0
	,, No. 7	0	0	0	0	0	0
Ox heart	,, No. 30	c	c	c	c	c	c
	,, No. 7	0	vm	c	c	c	c

M.H.D. of complement. 0.005 c.c.
Controls. — Serum alone with 3 doses of complement in saline solution = c.
Antigen alone with 3 doses of complement = c.
Complement control = 0.005 c.c.

As will be seen in Table X, the serum of rabbit No. 30 (Shiga immune serum) has fixed 18 doses of the complement in the presence of the saline emulsion of alcoholic extract derived from the guinea-pig heart, while the alcoholic extract of the ox heart lacked this property.

3. Precipitation with alcoholic Extracts.

It has been shown by Sordelli and Pico, Sachs and Guth independently that when the Forssman antibody and alcoholic extracts of a tissue which acts as the Forssman antigen are mixed, a precipitate formation is observed. Shiga immune serum was tested with this reaction also.

Methods.

Antisera.

1) Serum of rabbit No. 30 (Shiga immune serum, above described).
2) Serum of rabbit No. 7 (Forssman antibody, above described).
3) Serum of rabbit No. 17 (Forssman antibody, above described).
4) Serum of guinea-pig No. 40 (pure isophile antigoat haemolytic immune body, M.H.D. 0.002 c.c., drawn off 8 days after the third injection of washed goat red blood corpuscles).

Alcoholic Extracts.

For the Forssman antigen goat red blood corpuscles were used, and for the non-Forssman antigen those of man and ox. The methods for the preparation of alcoholic extracts were quite the same as mentioned above.

Procedures.

Two parts of each alcohol extract were evaporated in the incubator to one part. One part of the extract thus codensed was slowly mixed respectively with 5 parts of normal saline solution. Varying M.H.D of each antiserum were added to each of the tubes containing 0.5 c.c. of each saline emulsion. After incubation for about 18 hours at 37°C. the results were immediately examined in a warm place (###, ##, #, +, ±, —, respectively, indicate the intensity of the precipitation) (Table XI).

Table XI.

Number of antiserum	Red blood corpuscles of								
	Goat			Man			Ox		
	Amount of antiserum (M.H.D.)			Amount of antiserum (M.H.D.)			Amount of antiserum (M.H.D.)		
	50	30	10	50	30	10	50	30	10
Serum of rabbit No. 30	###	##	##	±	—	—	—	—	—
,, No. 7	###	###	###	±	—	—	—	—	—
,, No. 17	###	###	###	±	—	—	—	—	—
Serum of guinea-pig No. 40	—	—	—	—	—	—	—	—	—

Controls. — Emulsion controls, all negative.
Serum with saline solution containig the same amount of alcohol as the emulsions: negative.

As illustrated in Table XI, a distinct precipitate formation was only observed as the result of interacting the Forssman antibody as well as the Shiga immune serum with the saline emulsion of alcoholic extract of the goat corpuscles which act as the Forssman antigen.

The results of this section are summarised as follows: —

The goat haemolysin contained in the Shiga immune serum reacts specifically with known Forssman antigen in vitro.

III. Experiments with Cholera Vibrio.

A. Specific Reaction of Cholera Vibrio for the Forssman Antibody.

1. Fixation of the Forssman Antibody by Cholera Vibrio.

Methods.

Antisera.

1) Serum of rabbit No. 17 (Forssman antibody, already described).

2) Serum of rabbit No. 16 (antiox haemolytic immune body, already described).

Procedures.

Thirty milligrammes of an 18 hours culture of cholera vibrio were added to each of the tubes containing 1.0 c.c. of each antiserum diluted with saline solution (contains 100 M.H.D.). The tubes were put on the ice for one hour, and haemolytic doses, after centrifuging, were tested (Table XII).

Table XII.

No. of antiserum	Serum of rabbit No. 17.					Serum of rabbit No. 16				
c.c.	0.3	0.1	0.05	0.03	0.01	0.3	0.1	0.05	0.03	0.01
Amount of antiserum in M.H.D.	30	10	5	3	1	30	10	5	3	1
Treated	c	c	c	c	ac	c	c	c	c	ac
Not treated	c	c	c	c	ac	c	c	c	c	ac

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, and complement = 0.

As shown in Table XII, the cholera vibrio have had no influence upon the Forssman antibody (serum of rabbit No. 17) as well as the antiox haemolytic immune body (serum of rabbit No. 16).

2. Complement Fixation with watery Extracts of Cholera Vibrio.

Methods (Table XIII).

Antiserum.

Serum of rabbit No. 17 (Forssman antibody, already described).

Antigen.

Two milligrammes of a 24 hours culture of cholera vibrio were suspended in 1.0 c.c. of normal saline solution, the other procedures were quite the same as noticed above.

Procedures.

A quantity of 0.03 c.c. of the antiserum, native as well as treated with goat red blood corpuscles, was added to each of the tubes containing 0.5 c.c. of the antigen. The mixture was incubated for one and half hours at 37°C., along with ascending doses of complement. The further procedures were carried out as already mentioned.

Table XIII.

Antiserum 0.03 c.c.	Doses of complement				
	4	6	8	10	12
Native	d	c	c	c	c
Treated (control)	d	c	c	c	c

M.HD. of complement. 0.0075 c.c.

Control. — Serum alone with 4 doses of complement in saline solution = c.
Antigen along with 4 doses of complement. = c.
Complement control = 0.0075 c.c.

As illustrated in Table XIII, 5 doses of the complement were fixed at both series of the test. The fixed amount of the complement, as already explained, may be accounted for by the Wassermann reacting body which developed as a by-product of the Forssman antibody.

B. Are Cholera Vibrio Capable of Generating the Forssman Antibody in Vivo?

Four rabbits varying in weight from 2780 to 3580 grammes were used. Various amounts of vibrio (the minimum quantity being 0.5, the maximum 5.6 milligrammes) were injected 4 times. A small amount of blood was taken at varying intervals (3–7 days). And haemolysin for goat red blood corpuscles, as well as specific agglutinin for cholera vibrio were tested (Table XIV).

Table XIV.

Number of rabbit	Serum obtained after	Weight of rabbit (in gramme)	Amount of vibrio (in milligramme)	Interval of bleeding (in days)	Haemolysis						Agglutination
					Amount of antiserum (c.c.)						Titer of agglutinin
					0.09	0.07	0.05	0.04	0.03	0.02	Dilution of antiserum
No. 10	(Normal)	3580	—	—	c	c	c	ac	vm	m	20
	1. inj.	3670	0.50	3	c	c	c	ac	vm	m	160

No. 10	II. inj.	3760	1.00	4	c	c	c	c	ac	vm	1280
	III. „	3530	1.75	6	c	c	c	c	ac	vm	5120
	IV. „	3500	3.50	7	c	c	c	c	c	ac	5120
No. 24	(Normal)	3030	—	—	c	c	ac	vm	vm	m	10
	I. inj.	2880	0.50	3	c	ac	ac	ac	ac	vm	320
	II. „	3100	1.00	4	c	ac	ac	vm	vm	m	1280
	III. „	2940	1.75	6	c	ac	vm	m			10240
	IV. „	2950	3.50	7	c	c	ac	vm			10240
No. 32	(Normal)	2780	—	—	c	c	c	ac	ac	vm	10
	I. inj.	2530	0.70	4	c	c	c	ac	ac	vm	40
	II. „	2600	1.40	6	c	c	ac	ac	ac	vm	640
	III. „	2660	2.80	6	c	c	ac	vm	vm	m	10240
	IV. „	2660	5.60	6	c	c	ac	vm	vm	m	10240
No. 33	(Normal)	2950	—	—	d	d	d	d	d	tr	40
	I. inj.	2830	0.70	4	d	tr	tr	tr	0	0	160
	II. „	2750	1.40	6	tr	tr	tr	0	0	0	5120
	III. „	2730	2.80	6	tr	tr	tr	0	0	0	5120
	IV. „	2670	5.60	6	tr	tr	tr	0	0	0	10240

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, complement and saline solution = 0 and —.

As Table XIV shows, the specific agglutinin for cholera vibrio was distinctly developed as the result of injecting cholera vibrio into rabbits. By this treatment of the rabbits the normal immune body for goat red blood corpuscles has shown a certain diminution instead of an increase. In one case (rabbit No. 10), however, it seems as if a slight increase of the normal haemolysin had resulted. When we compare this increase with that which was proved by injecting Shiga dysentery bacilli into rabbits, there is a pronounced difference between the two.

The results in this chapter are summarised as follows: —

1) Cholera vibrio do not react with the Forssman antibody in vitro.

2) Cholera vibrio are incapable of generating the Forssman antibody in vivo.

IV. Experiments with Typhoid and Paratyphoid B Bacilli.

A. Specific Reaction of Typhoid and Paratyphoid B Bacilli for the Forssman Antibody.

1. Fixation af the Forssman Antibody by the Bacilli.

Antisera.

1) Serum of rabbit No. 17 (Forssman antibody, already described).

2) Serum of rabbit No. 16 (antiox haemolytic immune body, already described).

Procedures.

Thirty milligrammes of an 18 hours culture of the bacilli were added to each of two test tubes containing respectively 1.0 c.c. of each antiserum diluted with normal saline solution (contains 100 M.H.D.). The tubes were put on the ice for one hour. Haemolytic doses of the antisera thus treated, after centrifuging, were tested (Table XV).

Table XV.

Number of antiserum	Serum of rabbit No. 17					Serum of rabbit No. 16				
c.c.	0.3	0.1	0.05	0.03	0.01	0.3	0.1	0.05	0.03	0.01
Amount of antiserum in M.H.D.	30	10	5	3	1	30	10	5	3	1
Treated with typhoid bacilli	c	c	c	c	ac	c	c	c	c	ac
control (not treated)	c	c	c	c	ac	c	c	c	c	ac
Treated with Paratyphoid B bacilli.	c	c	c	c	ac	c	c	c	c	ac
Control (not treated)	c	c	c	c	c	c	c	c	c	c

M.H.D. of complement. 0.0075 c.c.

Controls for serum, corpuscles suspension, and complement = 0.

As will be seen in Table XV, typhoid and paratyphoid B bacilli are unable to react with the Forssman antibody as well as the antiox haemolytic immune body in vitro. The results agree with those of Forssman and Widén, and Tsuneoka.

2. Complement Fixation with watery Extracts of Typhoid and Paratyphoid B Bacilli.

Methods (Table XVI).

Antiserum.

Serum of rabbit No. 17 (Forssman antibody, already described).

Antigen.

One milligramme of a 24 hours culture of typhoid bacilli, and 4.0 milligrammes of a 24 hours culture of paratyphoid B bacilli were suspended respectively in 1.0 c.c. of normal saline solution, and the suspensions were heated for one hour at 60°C. Supernatant fluids, after centrifuging thoroughly, were used as antigen.

Procedures.

In the case of the antigen derived from typhoid bacilli 0.03 c.c. of the antiserum was used, and in that derived from paratyphoid B bacilli 0.05 c.c. In both cases untreated antiserum and that treated with goat corpuscles were used. The other procedures were exactly the same as those in the previous complement fixation tests.

Table XVI.

Antigen derived from	Antiserum	Doses of complement				
		4	6	8	10	12
Typhoid bacilli	Native	c	c	c	c	c
	Treated with goat red blood corpuscles	c	c	c	c	c
Paratyphoid B bacilli	Native	c	c	c	c	c
	Treated with goat red blood corpuscles	c	c	c	c	c

M.H.D. of complement. 0.0075 c.c.

Controls. — Serum alone with 4 doses of complement in saline solution=c.

Antigen alone with 4 doses of complement=c.

As illustrated in Table XVI, the Forrsman antibody (the serum of rabbit No. 17) has not fixed the complement in the presence of watery extracts derived from typhoid and paratyphoid B bacilli.

B. Are Typhoid and Paratyphoid B bacilli Capable of Generating the Forssman Antibody in Vivo?

In the case with typhoid bacilli 5 rabbits varying in weight from 1860 to 2060 grammes were used. Varying amounts of the bacilli (0.5–2.0 milligrammes) were injected from 1 to 4 times. In the case with paratyphoid B bacilli 4 rabbits (weight from 3220 to 3550 grammes) were injected 4 times. The amount of the bacilli varied from 0.5 to 4.0 milligrammes. In both cases a small quantity of blood was taken at varying intervals (4–7 days). Haemolytic doses on the test amount of goat red blood corpuscles, and contents of each specific agglutinin were examined (typhoid bacilli-Table XVII, paratyphoid B bacilli-Table XVIII).

Table XVII.

Number of rabbit	Serum obtained after	Weight of rabbit in gramme	Amount of bacilli (in milligramme)	Interval of bleedings (in days)	Haemolysis: Amount of antiserum c.c. 0.2	0.1	0.07	0.05	0.03	Agglutination: Titer of agglutinin. Dilution of antiserum.
No. 50	(Normal)	1860	—	—	c	vm	m	m	d	20
	I. inj.	1690	0.5	4	c	m	m	m	d	320
	II. „	1690	1.0	4	c	vm	m	m	d	1280
	III. „	1960	1.0	7	vm	m	d	d	d	2560
	IV. „	2130	2.0	7	ac	vm	m	m	d	5120
No. 51	(Normal)	2060	—	—	ac	vm	m	m	d	20
	I. inj.	1830	0.5	4	d	tr	tr	tr	tr	640
No. 52	(Normal)	1930	—	—	vm	m	m	d	tr	10

No. 52	I. inj.	1880	0.5	4	m	m	d	tr	tr	320
	II. ,,	1870	1.0	4	d	d	tr	tr	tr	640
No. 53	(Normal)	2050	—	—	m	m	m	m	d	10
	I. inj.	2130	0.5	4	m	m	m	m	d	320
	II. ,,	2080	1.0	4	d	d	d	d	d	1280
	III. ,,	1870	1.0	7	d	d	d	d	d	1280
No. 54	(Normal)	2000	—	—	c	vm	vm	m	d	20
	I. inj.	2220	0.5	4	vm	m	m	m	tr	640
	II. ,,	2080	1.0	4	vm	m	d	d	tr	1280
	III. ,,	1660	1.0	7	vm	m	m	d	tr	5120

M.H.D. of complement. 0.01 c.c.

Controls for serum, corpuscles suspension, complement and saline solution=0 and .

Table XVIII.

Number of rabbit	Serum obtained after	Weight of rabbit (in gramme)	Amount of bacilli (in milligramme)	Interval of bleeding (in days)	Haemolysis					Agglutination
					Amount of antiserum (c.c.)					Titer of agglutinin
					0.1	0.07	0.05	0.03	0.01	Dilution of antiserum
No. 34	(Normal)	3550	—	—	c	c	c	c	vm	20
	I. inj.	3200	0.5	4	c	ac	ac	vm	tr	640
	II. ,,	2770	1.0	6	c	ac	vm	vm	tr	2560
	III. ,,	2850	2.0	7	c	ac	vm	vm	tr	5120
	IV. ,,	3150	4.0	7	ac	vm	m	d	tr	10240
No. 35	(Normal)	3220	—	—	c	c	ac	vm	d	80
	I. inj.	3120	0.5	4	c	c	c	ac	d	320
	II. ,,	3040	1.0	6	c	c	ac	ac	tr	640
	III. ,,	3060	2.0	7	c	ac	ac	vm	tr	10240
	IV. ,,	3100	4.0	7	c	ac	vm	m	tr	10240
No. 36	(Normal)	3470	—	—	c	c	c	c	vm	10
	I. inj.	3350	0.5	4	c	c	c	c	vm	80
	II. ,,	3390	1.0	6	c	c	c	c	vm	640
	III. ,,	3400	2.0	7	c	c	c	ac	vm	5120
	IV. ,,	3500	4.0	7	c	c	c	ac	vm	10240
No. 41	(Normal)	3360		—	c	c	c	c	vm	80
	I. inj.	2900	0.5	4	c	c	c	ac	vm	320
	II. ,,	3030	1.0	6	c	c	c	ac	m	1280
	III. ,,	2840	2.0	7	c	c	vm	m	tr	10240
	IV. ,,	2000	4.0	7	c	c	vm	m	tr	10240

M.H.D. of complement. In the case of No. 34, 35. 0.01 c.c.

In the case of No. 36, 41. 0.0075 c.c.

Controls for serum, corpuscles suspension, complement, and saline solution=0 and .

As shown in Tables XVII and XVIII, a considerable quantity of the specific agglutinin was developed, but normal haemolysin for goat corpuscles seemed rather to decrease. The result of animal experiments with typhoid bacilli agrees with that of Forssman and Widén.

The results in this chapter are summarised as follows: —

1) Typhoid and paratyphoid B bacilli do not react with the Forssman antibody in vitro.
2) They are incapable of generating the Forssman antibody in vivo.

V. Experiments with Gärtner Bacilli.

A. Specific Reaction of Gärtner Bacilli for the Forssman Antibody.

Fixation of the Forssman Antibody by the Bacilli.

Methods.

Antisera.

1) Serum of rabbit No. 7 (Forssman antibody, already described).

2) Serum of guinea-pig No. 40 (pure isophile antigoat haemolytic immune body, already described).

3) Serum of rabbit No. 16 (antiox haemolytic immune body, already described).

Procedures.

Forty milligrammes of an 18 hours culture of Gärtner and paratyphoid B bacilli were added respectively to each of the tubes containing 1.0 c.c. of each antiserum diluted with saline solution (contains 100 M.H.D.). After one hour's incubation at 37°C., the tubes were centrifuged. A descending amount of the antisera thus treated was added respectively to several tubes containing 0.5 c.c, of a 3 per cent. saline suspension of goat or ox red blood corpuscles. After one hour's digestion in the ice-chest, the tubes were centrifuged and the supernatant fluid was pipetted off. Each sediment of corpuscles was resuspended in 1.0 c.c. of normal saline solution and haemolysis test was carried out (Table XIX).

Table XIX.

Treated with	Number of antiserum	Amount of antiserum in c.c. and M.H.D.				
		0.2 20	0.1 10	0.05 5	0.03 3	0.01 1
Gärtner bacill	No. 7	c	c	c	c	c
	No. 40	c	c	c	c	c
	No. 16	c	c	c	c	c

Paratyphoid B bacilli	No. 7	c	c	c	c	c
	No. 40	c	c	c	c	c
	No. 16	c	c	c	c	c
Control (not treated)	No. 7	c	c	c	c	c
	No. 40	c	c	c	c	c
	No. 16	c	c	c	c	c

M.H.D. of complement. 0.0075 c.c.
Controls for serum, corpuscles suspension and complement=0.

As shown in Table XIX, the suspensions of red blood corpuscles, which were sensitized with a single minimum haemolytic dose of the corresponding immune body, gave complete haemolysis. Thus, Gärtner bacilli reacted neither with the Forssman antibody nor with the other antisera in just the same manner as paratyphoid B bacilli.

B. Are Gärtner Bacilli Capable of Generating the Forssman Antibody in Vivo?

Three rabbits weighing 3030, 3545 and 3620 grammes respectively were used in this experiments. Various amounts of the bacilli (the minimum quantity being 1.0, and the maximum 7.0 milligrammes) were injected on each occasion from 1 to 4 times. A small amount of blood was drawn off at intervals of 6 or 7 days after the injections. Haemolysin for goat red blood corpuscles, as well as specific agglutinin for Gärtner bacilli were tested (Table XX).

Table XX.

Number of rabbit	Serum obtained after	Weight of rabbit (in gramme)	Amount of bacilli (in milligrmme)	Interval of bleeding (in days)	Haemolysis: Amount of antiserum (c.c.) 0.2	0.1	0.07	0.05	0.03	Agglutination: Titer of agglutinin Dilution of antiserum
No. 87	(Normal)	3030	—	—	ac	vm	vm	vm	vm	1280
	I. inj.	3000	1.0	6	ac	vm	vm	vm	vm	10240
	II. ,,	3050	2.0	6	ac	vm	m	m	m	10240
	III. ,,	2850	4.0	7	ac	vm	m	m	m	10240
	IV. ,,	2860	7.0	7	ac	m	m	m	d	10240
No. 84	(Normal)	3620	—	—	c	ac	vm	m	m	640
	I. inj.	3440	1.0	6	c	ac	vm	m	d	2560
	II. ,,	3490	2.0	6	c	vm	m	m	d	10240
	III. ,,	3520	4.0	7	c	vm	m	m	d	10240
	IV. ,,	3550	7.0	7	c	vm	m	d	d	10240
No. 85	(Normal)	3545	—	—	c	vm	vm	d	d	640
	I. inj.	2990	1.0	6	d	d	tr	tr	tr	10240

M.H.D. of complement. 0.0075 c.c.
Controls for serum, corpuscles suspension, complement, and saline solution=0 and —.

As will be seen in Table XX, a considerable quantity of the specific agglutinin has increased as the result of injecting Gärtner bacilli into the rabbits. On the other hand, the normal immune body for goat corpuscles showed rather a slight diminution instead of an increase.

The results in this chapter are summarised as follows: —

1) Gärtner bacilli do not react with the Forssman antibody in vitro.
2) They are unable to generate the Forssman antibody in vivo.

Rothacker reported on the occasion of research on meat poisoning, that antisera from 6 rabbits immunized with a mixture of paratyphoid B and Gärtner bacilli, showed a higher lytic action on sheep red blood corpuscles than sera from normal rabbits. According to my experiments, these two bacilli are unable to generate goat haemolysin in rabbits. Tsuneoka could not find any degree of fixation of the Forssman antibody by paratyphoid B bacilli either. It seems to me that some doubtful points are involved in the report of Rothacker. He did not define the kind of agar cultures. Moreover, he compared the haemolytic power of antisera from rabbits immunized with the mixture with that of serum from another normal rabbit. The horse meat contains a certain amount of the Forssman antigen. Thus, if broth made from horse meat is used for the cultures, a fallacy may be induced. On the other hand, it is a well known fact that normal immune body for sheep corpuscles contained in serum of normal rabbit is individually very variable. Therefore, it is absolutely necessary to examine, at the same time, with the same test materials all specimens from the same animal taken before and after treatment.

VI. Examination of Haemolysin for Goat red Blood Corpuscles Contained in Human Sera.

It is well known that human sera always contain a certain amount of haemolysin for sheep and goat red blood corpuscles. This haemolysin and the Forssman antibody, according to Friedemann and Taniguchi, are of the same nature from the serological point of view. Taniguchi has examined the haemolytic power of 134 human sera for 0.5 c.c. of a 3 per cent. suspension of sheep corpuscles, and reported that 6 were found to be very rich in the sheep haemolysin, one of which was so powerful as M.H.D. 0.003 c.c. Dünner (1917)[11] observed that one specimen out of 60 human sera was strongly haemolytic for sheep corpuscles, its minimum haemolytic dose was 0.004 c.c., tested with 0.5 c.c. of a 5 per cent. suspension. I have examined the haemolytic doses of 162 human sera on 0.5 c.c. of a 3 per cent. suspension of goat corpuscles (Table XXI).

Table XXI.

Number of serum	M.H.D. for 0.5 c.c. of 3 per cent. goat red blood corpuscles suspension (c.c.)	
162	0.1+	67
	0.1	13
	0.07–0.02	77
	0.01	4
	0.007	1

Controls for serum, corpuscles suspension, and complement=0.

As will be seen in Table XXI, out of 162 human sera, 5 were found to be very rich in goat haemolysin. Hence, it will be expected that a certain anaphylactic condition may develop as the result of injecting the Forssman antigen into human subjects where naturally a considerable amount of the corresponding antibody is present.

Conclusions.

1. Shiga dysentery bacilli contain the Forssman antigen; thus,
   1) they react with the Forssman antibody specifically in vitro; in detail, a) when the Forssman antibody is digested with the bacilli, the former is absorbed by the latter to a great extent, while another immune bodies (such as rabbit v. ox, guinea-pig v. sheep) suffer no change by this treatment; b) multiple doses of complement are fixed by the Forssman antibody in the presence of watery extract of the bacilli, after treating the antiserum with goat corpuscles a few being fixed in virtue of the Wassermann reacting body, which has developed as a by-product of the Forssman antibody;
   2) the bacilli lead to the development of thermostable goat haemolysin when injected into suitable animals (the rabbit type);
   3) the haemolysin thus generated should be considered as the Forssman antibody from the immunological point of view: the haemolysin reacts with the Forssman antigen specifically in just the same way as the Forssman antibody; in detail, a) the haemolysin is absorbed by saline suspension and alcoholic extract of a tissue which acts as the Forssman antigen; b) the haemolysin is capable of fixing complement and forming precipitate when the haemolytic antiserum is mixed with saline emulsion of the lipoids above described; c) whereas, the experiment either with the reagents derived from the non-Forssman antigen or with another immune bodies (such as rabbit v. ox, guinea-pig v, sheep) is negative;
   4) the haemolytic power of the haemolysin, like the Forssman antibody, is specific only for goat or sheep red corpuscles, any kind of group-and isohaemolysins being absent.

2. The goat haemolysin thus generated is an amboceptor responsible to the specific antigen contained in the bacilli, but it is quite distinct from the specific agglutinin; thus, it is as well absorbed by the bacilli as the agglutinin, while by treating with goat corpuscles the antiserum is never deprived of its agglutinating power.
3. Human sera contain a variable amount of the Forssman antibody; therefore, the conditions for the heterophile anaphylaxis or Forssman antibody anaphylaxis are provided when Shiga dysentery bacilli are injected into a human subject, where naturally a considerable amount of the Forssman antibody is present. The intensity of the anaphylactic intoxication would depend upon the amount of the antibody, which varies individually within a very wide range.
4. Cholera, typhoid, paratyphoid B and Gärtner bacilli do not contain the Forssman antigen; thus,
   1) They do not react specifically with the Forssman antibody in vitro;
   2) They are incapable of generating the Forssman antibody when injected into rabbits.

In conclusion, let me say that no words can adequately express my deep sense of appreciation for Director Nagayo's continual encouragement; at the same time, this small effort on my part is due in a great measure to Professor Hayashi, Professor Mita and Dr. Taniguchi, who have at all times given me very useful advice and help.

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Studien über das Hämolytische Komplement, insbesondere zur Ersatzbarkeit seiner einzelnen Komponente

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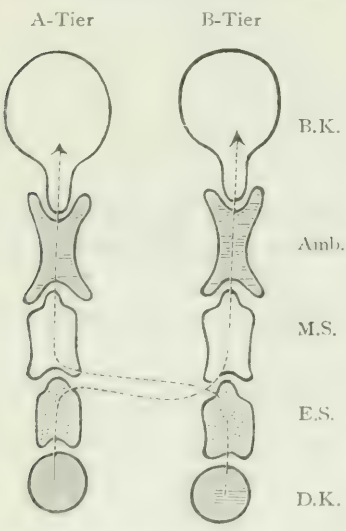
Inhaltverzeichniss.

I. Einleitung.
II. Eigene untersuchungen.
  1. Das Gehalt vom Komplement und Normal-ambozeptor der verschiedenen Lebwesen; zum Anhang die Schwankungsbreite des Komplementtiters bei der Schwangerschaft.
  2. Die Ersatzbarkeit zwischen jeder Komplementstücke in den verschiedenen Warmblütern.
  3. Die Ersatzbarkeit zwischen jeder Komplementstücke der Warmblütern und Geflügern.
III. Schlusssätze.

I. Einleitung.

Es ist im Allgemeinen bekannt, dass das Komplement von dem immunoserologischen Standpunkte aus, eine grosse Bedeutung zeigt. Daher ist es, seit der ersten Anerkennung des Komlementes auf unseren Fachkreis, recht zahlreiche vom verschiedenen Gesichtpunkte aus beobachtete Arbeit erschienen, so dass es schwer ist, nach ihrer umfangreichen literalischen Entwickelung zu verfolgen. Wir kennen bereits, dass die Komplemente in dem verschieden Lebwesen weit ausgebreitet vorhanden sind. Jedoch scheint es noch zweifelhaft zu sein, dass solche Komplemente alles als eine ganz gleiche Substanz betrachtet werden können. Vorausgesetzt, dass es hier ein mit Ziegenblutkörperchen vor behandeltes, inakti-

viertes Kaninchenimmunserum (Ambozeptor) gibt. Lässt man nun zu diesem Immunserum frische Sera vom verschiedenen Ursprung, d. h. das Komplement einwirken, so erkennt man, dass dies Immunserum wieder sich reaktivirt. Auf Grund dieser Tatsache können wir wohl vermuten, dass die Komplemente welche in den verschiedenen Lebwesen weit vorhanden sind, logisch als eine gleiche Substanz betrachtet werden. Jedoch scheint es mir, dass solche Auffassung von der gegenwärtigen Wissenschaft schwer anerkannt werden. Wollte man hier zu den Beweis führen, dass die Komplemente im verschiedenen Lebwesen von gleicher Substanz sind, so ist es notwendig solche Komplemente zuerst analytisch zu untersuchen. Wird ausgedruckt im anderen Worte, so müssen die jeden Komplemente zuerst in „Globulin" (Mittelstück) und „Albumin" (Endstück) zerlegt, und dann weiter beobachtet werden, ob das Mittelstück vom A-Tier, mit dem Endstück vom B-Tier, ferner noch das Mittelstück vom B-Tier, mit dem Endstück vom A-Tier sich gut verbindet und damit ein Komplement gebildet werden kann oder nicht. Wird ferner noch mit anderem Worte ausgedruckt, so müssen wir es bestätigen, ob das Endstück vom A-Tier durch dasselbe vom B-Tier, ebenfalls das Endstück vom B-Tier durch dasselbe vom A-Tier, und schliesslich noch weiter das Mittelstück vom A-Tier durch dasselbe vom B-Tier, ebenfalls das Mittelstück vom B-Tier durch dasselbe vom A-Tier alles in gekreuzter Weise sich verbinden können oder nicht (Vergl. die unten stehende Figur).



B. K. = Blutköperchen
Amb. = Ambozeptor
M. S. = Mittelstück
E. S. = Endstück
D. K. = Drittes Komponent

Wenn solche Komplizierten Wechselbezichungen zwischen jeden Komponenten in den verschiedenen Komplementen nicht nur im dem Komplemente von Warmblütern allein, sondern auch in demselbe von den verschiedenen Lebwesen klar gemacht werden können, so können wir wohl sagen, dass die Gleichheit der Komplemente in den verschiedenen Lebwesen zunächst besteht.

Die unten stehende Figur bezeichnet sich eine Zerlegung des Komplementes vom A- und B-Tiere, und wird klar gemacht, wie das Mittelstucks vom A-Tier mit dem Endstück vom B-Tier und umgekehrt das Mittelstück vom B-Tier mit dem Endstück vom A-Tier in gekreuzter Weise sich verbindet.

Kurz gesagt, müsste meine Untersuchung eine Beziechung zur Ersatzbarkeit der jeden Komplementstücke von den verschiedenen Lebwesen klar gemacht werden, damit die Gleichheit jeden Komplemente versichert werden könnte.

III. Eigene Untersuchungen

Hier zunächst wird unten die Untersuchungsordnung erwähnt:

(1) Das Gehalt von Komplement und Normal-Ambozeptor der verschiendenen Lebwesen; zum Anhang die Schwankungsbreite des Komplementtiters bei der Schwangerschaft.

(2) Die Ersatzbarkeit zwischen jeder Komplementstücke in den verschiedenen Warmblütern

(3) Die Ersatzbarkeit zwischen jeder Komplementstücke in den Warmblütern und geflügeln

(4) Die Ersatzbarkeit zwischen jeder Komplementstücke in den Warm- und Kaltblütern

(5) Die Ersatzbarkeit zwischen jeder Komplementstücke in den verschiedenen Geflügeln und Kaltblütern.

1. Das Gehalt von Komplement und Normalambozeptor der verschiedenen Lebwesen, zum Anhang die Schwankungsbreit des Komplementtiters bei der Schwangerschaft.

Diese Untersuchung ist zunächst als Vorprüfung ausgeführt worden, um die nacher in der Rede stehende Ersatzbarkeit zwischen jeden Komplementstücke klar zu machen.

Technik der Untersuchungen.

Als die Untersuchungsmethode ist die Hämolyse angewendet worden. Zunächst werden die in der beliebiger Weise entnommenen verschiedenen frischen Sera (Komplemente) in die Reagenzröhrchen hineingetan — 0.01–0.03–0.04–0.05–0.06–0.07–0.08–0.09–0.1 cc. —; und dazu hämolytischer Ambozeptor (Anti-ziegenblut-Kaninchenambozeptor, Gebrauchdosis 0.005) und 5% Ziegenblutaufschwemmung 0.5 c.c. (3 mal gut ausgewascht und suspensiert in physiologischer Kochsalzlösung in 5%, welche gerade entsprechend dem 5% der Ziegenblutdichte), hintereinander zugesetzt, und schliesslich das Inhalt Volum der jeden Reagenz-Röhrchen durch Hinzufügung der physiologischen Kochsaltlösung gleich gemacht. Worauf werden die jeden Reagenzröhrchen gut geschüttert und dann 2 Stunden lang in dem Brutofen (32°C.) liegen gelassen und nachher im Eiskammer bis zum Morgen ruhig gestellt. Die hämolytische Stärke wurde immer am nächsten Morgen beobachtet.

Dabei alle nötige Komtrollversuche sind natürlich nicht vernachlässigt worden. Wenn die frischen Sera Normalambozeptor enthalten, so mussten dieselben im voraus immer durch has Kälte-Trennungsverfahren von Ehrlich u. Morgenroth beseitigt werden.

Als die untersuchenden Materialien wurden hier zunächst die folgenden

Arten von Sera ausgewählt; d. h. die Sera von Meerschweinchen, Kaninchen, weissen Ratten, Wild- und Hausratten, Katzen, Schweinen, Hunden, Menschen, Pferden, Rinder, Ziegen, Hammel, Hühnern, Tauben, Enten, Ochsenfrosch (japanisch "Gama" genannt), Fröschen, u.s.w.

Jedoch werden hier einzelne Untersuchnngs-Ergebnisse von jeden Materialien nicht näher geschildert, sondern gleich kurz zusammenfassend darauf besprochen;

(1) Komplementgehalt vom Meerschweinchenserum zeigt nur ganz geringe individuelle Schwankung, namentlich nach dem Unterschied von Geschlecht, Körpergewicht und nach der Haarlänge und Farbe, etc. Die minimale hämolytische Dosis von erwachsen Meerschweinchen in den meisten Fällen 0.04, nicht selten 0.02–0.03. Es war nur ein einziger unter 20 Untersuchungsfällen, welcher die minimale hämolytische Dosis höchstens 0.08 zeigte.

(2) Das Serum von Meerschweinchen-embryonen enthalten kein Komplement; dasselbe tritt zuerst an 3–4 Tagen nach der Geburt auf. Von diesem Lebensalter steigt sich das Komplementgehalt immer höher; am 9–10 Tagen nach der Geburt erreicht es jedoch etwa ein Sechstel von dem erwachsenen. Trotzdem in dem 15–20 tägigen Neugeborenen war die minimale hämolytische Dosis nur auf 0.05–0.07 erreicht.

Die Tatsache, dass in den Embryonensera kein Komplement nachgewiesen wird, sind mit dem von Obata[1] an Menschenmaterialien angestellten Resultat ganz vereinigt. Dass, das erste Auftreten des Komplements in den Meerschweinchen bedeuted früher bemerkt wird, als beim Menschen (erst im 8. Monate erschienen), wird als einen grossen Unterschied bei der Komplemententwicklung in den Tieren und Menschen angesehen. Ferner die Tatsache, dass die Embryonen- und Neugeborenensera gegen Ziegenblutkörperchen nicht hämolytisch einwirken, ist mit dem von Obata[1] an den Menschenmaterialien beobachteten Ergebnisse vollkommen entsprochen.

(3) Das Komplementgehalt der Neugeborenen von den Meerschweinchen (4–14 Tage alt), ist viel geringer als dasselbe von ihren Müttern. Es zeigte sich die minimale hämolytische Dosis 0.08–0.09, während dieselbe von den Muttersera etwa 0.06 zeigte. In der weiteren Entwicklung (Im Alter von 15–30 Tagen) lässt sich das Komplementgehalt der Neugeborenen im Vergleich von den Muttersera nicht so beträchtlich unterscheiden.

(4) Vor der Geburt d. h. wahrend der Schwangerschaft wurde in der Mehrzahl der Fälle keine Komplementverminderung bemerkt, ausgenommen davon, dass in den seltenen Fällen ein relativ geringeres Komplementgehalt (0.08) beobachtet wurde. Im Gegenteil wurde das Komplement nach der Geburt besonders etwa an 4 Tagen im Allgemeinen vermindert. In den von mir untersuchten 14 Fällen war nur in einem das Komplementgehalt 0.09, während die anderen die minimale hämolytische Dosis zwischen 0.06–0.08 sich schwankten. Im weiteren Zeitverlaufe nach der Geburt erholt sich sie allmählich. Solche Ergebnisse, dass das Komplement im gewissen Zeitraum nach der Geburt sich vermindert und in

weiteren Verlaufe wieder sich erhöht, scheinen mit der Ermüdung durch die Geburt und mit der Erholung der physiologischen Vorgänge des Mutterkörpers sich beziehen. Meine Resultate stimmen wohl hiermit mit denselben von Lüdke[3] (bei Kaninchen) und Santschenko[4] (bei Menschen) überein.

(5) Die hämolytische Fähigkeit der Meerschweinchensera gegen das Ziegenblutkörperchen ist ziemlich selten nachgewiesen. Verfasser hat es nur 5 mal unter 45 Untersuchungsfällen beobachtet. Dass, es durch echte Hämolysine geschieht, ist durch die Kältetrennungs- und Inaktivierungsmethode sicher bestätigt worden. Daher lässt es mir zweifeln, warum *Inoki*[27] in seinen zahlreichen Fällen niemals solches bemerkt hatte.

(6) Das verhältniss des hämolysins zur Schwangerschaft. Von dem Freigebniss, dass in einem einzigen Fall von mir untersuchten Sera von 14 tragenden Meerschweinchen eine beträchtlich grosse Dosis vom Hämolysin enthalten war und fernerhin in einem tragenden weissen Rattenserum auch ein hoches hämolysingehalt (Titel: 80X) nachgewiesen wurden, lässt es sich mir wohl bedenken, dass es zwischen der Schwangerschaft und Erhöhung des Hämolysingehaltes wahrscheinlich eine gewisse Wechsel-Beziehung vorhanden sein muss.

(7) Das Komplementgehalt des Kaninchenserums; Es ergibt sich hierbei viel deutlichere individuelle Schwankung zum Vergleich mit dem Fall vom Meerschweinchen. Unter 20 Fällen liesen sich in 3 ganz spurweise Komplementwirkung nachweisen, indem die minimale hämolytische Dosis in einem Fall 0.05, in 11 0.06, in 1 0.07 und schliesslich in 5 0.09 d.h. im Mittel etwa 0.07 zeigte. Das aktive Kaninchenserum wirkt fast immer gegen das Ziegenblut Hämolytisch ein. Sein Hämolysingehalt ist relativ gross, ganz selten Titel über 20X bemerkt, jedoch der Hämolysintitel gewöhnlich von 5 bis 10X sich schwankte.

(8) Das Komplementgehalt des weissen Rattenserums: Frisches weissen Rattenserum enthält das gegen die Ziegen-, Hammel-, Meerschweinchen- und Kaninchenblutkörperchen etc einwirkende Heterolysin, während die Rinder-Pferde- und Menschenblutköperchen nie davon angeriffen werden. Das Titel vom Heterolysin ist ziemlich wechselnd. Unter 18 Fällen lassen sich nur in 2 Fällen fast kein Hämolysin nachweissen. Gewöhnlich stellt sich der Hämolysin-titel von 5 bis 6X, selten 10X dar. Nur in einem einzigen Fall von den tragenden Weissen-Ratten erreichte das Hämolysin-titel bis 80X. Das war einer der Gründe, dass der Verfasser zum Verhältniss des Hämolysingehaltes zur Schwangerschaft eine Frage gestellt hat. Das Komplementgehalt der weissen Ratten ist auch ziemlich schwankend. In 2 von 18 Untersuchungsfällen erreichte die minimale hämolytische Dosis kaum bis 0.1, während die anderen 8 Serien 0.04–0.05 und die ubrigen 3 0.07–0.08 und endlich noch 2 nur 0.1 zeigten. Von der oben erwähnten Tatsache, dass das weisse Rattenserum viel Hämolysin enthält und ein ziemlich wechselndes Komplementgehalt zeigt, lässt es sich praktisch schwer verwerten.

(9) Das Komplementgehalt des wild- und Hausrattenserums (Mus Deccumanus, Mus Alexandrinus, und Mus rattus); Unter 3 Arten von diesen Ratten ist das

Komplementgehalt von M. Decc. am grössten, d. h. die minimal hämolytische Dosis in der Mehrzahl erreichte auf 0.06, ganz selten 0.1, während sie von Mus Alex. in den meisten Fällen 0.07 und bei Mus Rattus höchstens 0.08–0.09 zeigten. Bei dem letzteren finden sich ganz selten Fälle, bei welchen nur ein Spur vom Komplement nachgewiesen war. Das Hämolysingehalt von Mus Decc. und von Mus Alex., besitzt fast immer gleiches Titel (Titel: 10), dagegen dasselbe von Mus rattus höchstens 5X erreichte. Schliesslich besitzte das frische Serum solcher Ratten gegen das Ziegen-, Hammel-, Kaninchen- und Meerschweinchen-Blutkörperchen auch hämolytisch wirkende Eigenschaft, wie es bei den weissen Ratten gesehen war.

(10) Das Komplementgehalt der Katzenserums ist fast immer in 0.06–0.07, ganz selten 0.09–0.1. Das frische Katzenserum löst auch das Ziegen- und Hammelblutkörperchen im leichten Grad auf (Hämolysin-titel 5–6X, ganz selten 10X).

(11) Das Komplementgehalt des Schweinserums; Dasselbe stellt sich eine relativ leichte individuelle Schwankung dar. Die minimale hämolytische Dosis ist in der Mehrzahl in 0.05–0.06, ganz selten in 0.08–0.1. Das frische Schweineserum löst auch das Ziegen- und Hammelblutkörperchen ziemlich deutlich auf; ihr Hämolysintitel ziemlich hoch, etwa doppelt so hoch zum Vergleich mit dem von Kaninchen (in den meisten Fällen 20X. selten 40X). Von diesem Grunde ist das Schweinekomplement zur praktischen Anwendung leider wenig zweckmässig, ob wohl das Komplementgehalt bei diesen Tieren ziemlich reichlich ist und zwar keine erheblich individuelle Schwankung zeigt.

(12) Das Komplementgehalt des Hundserums: Dasselbe schwankt sich auch individuell ganz wenig. Gewöhnlich ist die minimale hämolytische Dosis in 0,06–0.07, selten 0.05. Das frische Hundserum löst das Ziegen- und Hammelblutkörperchen ganz leicht auf (Hämolysintitel höchstens 4–5X, selten fast ganz negativ).

(13) Das Komplementgehalt vom Menschenserum; 25 Untersuchungsfälle vom 19–40 Jahren alt, darunter 15 Männer, 10 Frauen. Dasselbe stellt sich in den meisten. Fällen die minimale hämolytische Dosis 0.06–0.07, selten 0.05 dar. Unter 25 Untersuchungsserien waren nur 3 Fälle, bei welchen das Komplementtitel nur in 0.08 war. Mein Resultat stimmt mit demselbe von Gay und Ayer gar nicht überein, die des Komplementgehalt von Frauen zum Vergleich mit dem von Männern als grösser beobachteten. Bei den von mir untersuchten 19–40 jährigen Personen liess sich das Komplementtitel nach dem Unterschied vom Lebensalter keine merkliche Schwankung zeigen. Das frische Menschenserum löst in der Mehrzahl der Fälle das Ziegen- und Hammelblutkörperchen leicht auf. Diese Ergebnisse stimmen mit der Ansicht von Obata[1] gar nicht überein, nach dem das Frauenserum in doppelt so viel Fällen das Ziegenblutkörperchen löste, zum Vergleich mit demselbe von den Männeren. Das Hämolysintitel vom Menschenserum schwankt sich nach der individuellen Verschiedenheit ziemlich deutlich, besonders bei den Männersera. Im Allgemeinen erreichte das Hämolysintitel bis auf 10–20X.

(14) Das Komplement des Pferdeserums: Mit gewöhnlicher Methode fiel es dem Verfasser ganz schwer, im Pferdserum das Komplement nachzuweissen. Setzt man aber zum frischen Pferdeserum eine gewisse Menge vom Endstück des Meerschweinchenkomplements zu, so tritt dann eine ganz deutliche Komplementwirkung auf, während das eine gewisse Menge vom Meerschweinchenmittelstück zugesetzte Pferdeserum gar keine Komplement-Fähigkeit darstellen konnte. Von dieser Tatsache Kann man wohl verstehen, dass im Pferdeserum das Endstück vom Komplement fehlt, oder höchstens nur ganz wenig davon enthalten sei. Nach Inoki ist das Komplementtitel vom Pferdeserum etwa $\frac{1}{150}$ von demselben eines Meerschweinchens entsprochen; daraus schliesst er sich, dass es noch zu frühzeigtig ist, in der Weise aufzufassen, dass es im Pferdeserum das Endstück fehlt. Seiner Meinung stimme ich leider nicht überein. Man kann natürlich im Pferdeserum, wenn eine grosse Menge vom demselbe, z. B. 1.0–2.0 gebraucht wird, ohne den Zusatz vom Meerschweinchenendstück eine ganz spurweise Komplementwirkung nachweissen. Jedoch ist es damit selbstverständlich schwer beantwortet, dass man im Pferdeserum sicher Komplement nachweissen konnte. Im Gegenteil, wenn eine gewisse Menge vom Endstück zum Pferdeserum sich einwirkt so lässt das letztere mit der kleinen Menge, etwa 0.02–0.03 noch eine deutliche hämolytische Wirkung hervorrufen. Von diesem Grunde unterliegt es kein Zweifel mehr, dass im Pferdeserum Komplementendstück fehlt. (Vergl. Tafel I.).

(15) Das Komplement vom Rinderserum: Im Rinderserum fehlt auch das Endstück, ganz gleich, wie im dem Fall vom Pferde ist. Das Fehlen der Endstücke vom Pferd- und Rinderkomplement wird nicht nur durch das Zusetzen vom Meerschweinchenendstück, sondern auch durch dasselbe vom Kaninchen, Schweinen, Menschen, Ratten, Katzen, Hunden etc. ersetzt werden kann. Schliesslich enthält das Rinderserum, wie das Pferdserum sehr spärliches Hämolysin, dessen Titel höchstens 2–3X erreicht.

(16) Der Komplementtiter vom Ziegen- und Hammelserum erreicht höchstens auf 0.08–0.09.

(17) Das Komplement vom Geflügel: (a) Das Komplementgehalt von den Hühnern zeigt eine relativ wenig individuelle Schwankung zum Vergleich mit demselbe von den Tieren. Die minimale hämolytische Dosis liegt in der Mehrzahl der Fälle zwischen 0.07–0.08. (b) Das Taubenkomplementgehalt ist auf 0.7–1.0, d. h. das Titel von etwa $\frac{1}{10}$ von demselbe von den Hühnern. Wird aber dem Taubenserum eine gewisse Menge vom Hühner-endstück zugesetzt, so ruft das letztere mit viel geringerer Menge 0.09–0.1, noch eine merkliche hämolytische Wirkung hervor. Daraus wird es auch hier bestätigt, dass das Taubenkomplement auch an Endstück sehr spärlich ist. Schliesslich wird das Hühner- und Taubenkomplement in 53°C–30′, vollkommen inaktiviert.

(18) Das Komplement vom Kaltblüter: (a) Das Komplementtitel vom Ochsenfrosch schwankt sich zwischen 0.07–0.08, d. h. das Titel von etwa $\frac{1}{2}$ vom Meerschweinchen. Das frische Ochsenfroschserum löst das Ziegenblutkörperchen ganz leicht

Tafel I.

No:	Zutaten	A										B										C			
	5% Z. B. A.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Amb. 0.05	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
	Pferdserum	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1				
	Meerschw. M.S.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0											1.0		1.0	
	Meerschw. E.S.											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0			1.0	1.0
	0.85% Nacl-lös	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	—	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	—	2.5	1.0	1.0	1.0
1	Resultat	—	—	—	—	—	—	—	—	—	—	—	S	++	++	+++	+++	+++	+++	+++	+++	—	—	—	+++
2	„	—	—	—	—	—	—	—	—	—	—	—	—	+	++	++	++	+++	+++	++++	+++	—	—	—	+++
3	„	—	—	—	—	—	—	—	—	—	—	—	—	S	+	++	+++	+++	+++	++++	+++	—	—	—	+++
4	„	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S	+	++	+++	+++	—	—	—	+++
5	„	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S	+	+	++	++	+++	—	—	—	+++

Z. B. A = Anfschwemmung d. Ziegenblutkörperchen. Amb = Ambozeptor. M. S. = Mittelstück. E. S. = Endstück. Nacl = Kochsalz. +++ = Komplete Hämolyse. +++ = fast komplete Hämolyse. ++ = Mittelstarke Hämolyse. + = Schwache Hämolyse. S = purweise Hämolyse. — = Hämolyse negativ.

auf. (b) Das Komplementgehalt des Frosches ist etwas höher als dasselbe vom Ochsenfrosche, d. h. die minimale hämolytische Dosis etwa in 0.06–0.07. Endlich wird auch das Ochsenfrosch- und Froschkomplement in 42–45°C–30′, vollkommen inaktiviert.

(19) Die Komplemente von den oben erwähnten Warmblütern, Geflügeln und noch Kaltblütern zergliedern sich in zwei Komponenten, in das Mittel- und Endstück (Vergl den III. Teil).

2. Die Ersatzbarkeit zwischen jeden Komplementsstücke in den verschiedenen Warmblütern.

Im Laufe von dem jahre 1907–1911 wurde es von Ferrat[11] Altmann u. Sachs[12] und Liefmann[13] etc. mit ihrer spezifischen Methode nachgewiesen, dass das hämolytische Komplement in zwei Komponenten, — „Mittel- und Endstück" — sich zergliedert. Diese interessante Entdeckung ist gleich nachher recht von zahlreichen Autoren, namentlich Marks,[14] Sachs u. Bolkowska,[15] Braun,[16] Fränkel,[17] Guggenheimer,[18] Landsteiner,[19] Ritz,[20] Hecker,[21] Muttermilch,[22] Husler,[23] Amako,[24, 25, 26] Inoki,[27] Watabiki etc. nachgeprüft worden. Sie haben sich es denn nicht nur mit dem Komplement vom Meerschweinchen allein, sondern auch mit demselbe von den anderen Tierarten beschäftigt. Im jahre 1912 ist ferner noch von Ritz[27] im Komplement ein anderes Komponent angegeben worden, welches durch die Einwirkung von Cobragift geschädigt wird. Dieses letztere Element ist von Ritz als „drittes Komponent" genannt, deren Exsistenz heutzutage schon von mehreren Autoren (in Japan von Inoki,[27] Kanamori[28] eingehend untersucht) anerkannt ist. Hier werde ich daher in die weitere Erörterung dieses dritten Komponents nicht mehr eingehen, sondern werde ich erst später in einer anderen Gelegenheit davon reden. Dass, das Komplement von den verschiedenen Lebwesen, wie oben schon klar gemacht ist, vom verschieden Gehalt ist, und ferner noch, dass die Existeuz von zwei Komponenten durch die oben vorgestellten Autoren und das Verfasser selbst (III. Kapitel verglichen) sicher bestätigt sind, ist für meine Hauptuntersuchung „die Ersatzbarkeit zwischen jeden Komplementstücken" eine bedeutend wichtige Vorbereitung.

Der Hauptpunkt von meiner Untersuchung begreift sich wie folgendes;

Ob das Mittelstück vom B-Tier mit dem Endstück von A-Tier eine Komplementwirkung zeigt oder nicht, d. h., ob das Endstück vom B-Tier durch dasselbe vom A-Tier ersetzt werden kann oder nicht, und auch ob das Mittelstück vom A-Tier ebenfalls durch dasselbe vom B-Tier vertreten werden kann oder nicht? Mit anderen Worte ausgedrückt, ob ein Komplementstück vom A-Tier mit einem andern Komplementstück vom B-Tier ein Komplement konstituirten kann oder nicht? Weiter kurz gesagt, ob jede Komplementbestandteile von verschieden Lebwesen in gekreuzter Weise sich einander verbinden können oder nicht? Obwohl solche Frage bischer bereits von einigen Autoren (Liefmann u. Stutzer,[29] Marks,[30] Fränkel,[31] Landsteiner,[32] Inoki,[27] Watabiki, etc.) angestellt wurde, so ist es darüber

noch wenig befriedigend beantwortet und liegt es noch hierin viel Räume frei, welche noch genauer erforscht werden müssen.

Eigene Untersuchung und ihre Methodik.

Als die Methode von Komplementspaltung finden sich hier das Dialysierverfahren von Ferrata,[16] die Salzsäurefällung von Altmann-Sachs[17] und die Kohlensäurefällung von Liefmann.[18] Nach meiner Erfahrung ist die erste sehr zeitraubend und wenig folgrecht, und die zweite ist nicht so Zeitspielig, aber sein Resultat kann leider nicht immer sicher eruirt werden. Schliesslich ist die dritte Methode von Liefmann relativ einfach ausführbar, und sein Resultat ist im Vergleich mit den anderen bedeutend besser. Von diesem Grunde habe ich die Liefmann'sche Methode angewendet. Ich habe jedoch meiner Erfahrung nach einbisssen in folgender Weise modifiziert.

Zunächst wird das zu untersuchende Serum (Komplement) 2.0 cc. in mitteldickes Reagenzröhrchen eingenommen und dann mit 8.0 cc. von sterilisierten Aq. dest. verdünnt (also in 4 Fach verdünnt). Dann wird zu dieser Mischung das Kohlensäuregas (nach Kipp'schem Apparat) in 20–40 minuten lang eingeleitet, bis das verdünnte Serum sich stark opalesziert und reichlicher Niederschlag darin abgeschieden wird (Die Länge vom CO_2-Einleiten nach der Gas-Menge und nach der Verdünnung, dem Grade ferner noch nach der Verschiedenheit der untersuchenden Sera beliebig und verschieden geändert).

Die dadurch Gas eingeleitete Komplementlösung wird gleich in ein Spitzglas übergetragen und mindestens 30 minuten lang stark zentrifugiert (Tournenzahl über 3000X). Zu dem Supernant (der klare Oberteil) wird 10% Kochsalzlösung 0.85 c.c. zugesetzt und darauf noch physiologische Kochsalzlösung hinzugefügt, bis die ganze Menge sich auf 20 c.c. erreicht. Dieses Superanant wird weiterhin mit Tonfilter (Reichert) filtriert: der hiermit hergestellte Teil ist sog. „Endstücklösung." Nun wird der zentrifugierte Niederschlag mit sterilisiertem Aq. dest. mindestens 2 mal ausgewascht und schliesslich in 20 c.c. von physiogischer Kochsalzlösung aufgeschwommen; dieses ist sog. „Mittelstücklösung".

Die Mischung von der Mittelstück- und Endstücklösung, je 1.0 : 1.0 c.c. ist beinahe der Fähigkeit des Komplements 0.1 c.c. entsprechend.

Bei der Ausführung der Untersuchungen lassen sich nach dem Prinzip der hämolytischen Mechanismus dem Blutkörperchen zuerst der Ambozeptor dann das Mittelstück vom B-Tier, darauf das Endstück von A-Tier nacheinander einwirken, und schliesslich wird die Menge jeder Reagenzröhrchen mit 0.85% Kochsalzlösung bis zum gleichen Volum gemacht. Als Kontrollversuche wurden die folgenden Bedingungen immer in Acht genommen;

(1) Die Endstücklösung allein sich nie Hämolyse hervorruft,
(2) Die Endstücklösung mit dem Ambozeptor sich nie Hämolyse hervorruft,
(3) Die Mittelstücklösung allein sich nie Hämolyse hervorruft,
(4) Die Mittelstücklösung mit dem Ambozeptor sich nie Hämolyse hervorruft.

(5) Ob die Mittelstück- und Endstücklösung eines Tiers in seinem Zusammentreffen Hämolyse hervorruft oder nicht, d.h. ob das Komplement eines Tiers in zwei Komponente zerglierdert ist oder nicht.

Als die untersuchenden Tiere kamen die folgenden 12 Arten in Anwendung; Meerschweinchen, Kaninchen, Schweine, Katzen, Hunde, Menschen, weisse Ratten, Mus Deccumanus, Pferde, Rinder, Ziegen, Hammel, etc. Um die Verlegenheit bei den Untersuchungen zu vermeiden, sind die ganzen Experimente nach den folgenden Reihen ordentlich durchgeführt worden; (sehe Figuren)

Die oben in den Kreisen angegebenen wörter begreifen sich folgendes;

M. K. = Meerschweinchen-Komplement,
K. K. = Kaninchen-Komplement,
S. K. = Schweine-Komplement,
Ka. K. = Katzen-Komplement,
H. K. = Hunde-Komplement,
Men. K. = Menschen-Komplement,
w. R. K. = weisse Ratten-Komplement,
D. K. = Deccumanus-Komplement,
P. K. = Pferde-Komplement,
R. K. = Rinder-Komplement,
Z. K. = Ziegen-Komplement,
Ha. K. = Hammel-Komplement,

Nach den oben erwähnten Ordungen habe ich wohl über 200 Mal Experimente durchgeführt. Da die einzelnen Resultate von jeden Experimenten hier anzugeben schwer sind, so sei es nur erlaubt, unten mit dem Tafel Zusammenfassend verzeichnen zu lassen.

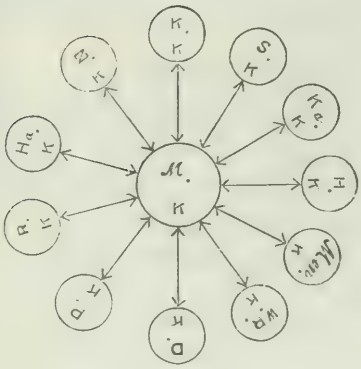
1. Meerschweinch. + M. S.

Kaninchen = E. S.	Komplementwirkung	ziemlich stark
Schweine = E. S.	,,	,, ,,
Katzen = E. S.	,,	schr schwach od. negativ
Hunde = E. S.	,,	,, ,,
Menschen = E. S.	,,	ziemlich stark
w. Ratten = E. S.	,,	,, ,,
Ziegen = E. S.	,,	schr schwach od. negativ
Hammel = E. S.	,,	,, ,,
Pferde = E. S.	,,	negativ
Rinder = E. S.	,,	,,

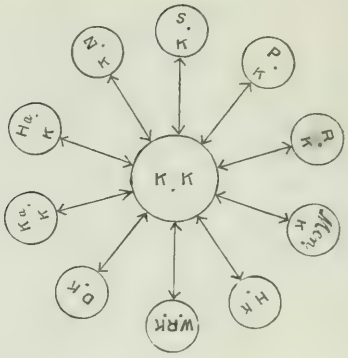
2. Meerschweinch + E. S.

Kaninchen = M. S.	Komplementwirkung	meist stark
Schweine = M. S.	,,	,, ,,
Katzen = M. S.	,,	,, ,,
Hund = M. S.	,,	,, ,,
Menschwn = M. S.	,,	,, ,,
Ratten = M. S.	,,	,, ,,
Ziegen = M. S.	,,	schwach
Hammel = M. S.	,,	,,
Pferde = M. S.	,,	ziemlich stark
Rinder = M. S.	,,	,, ,,

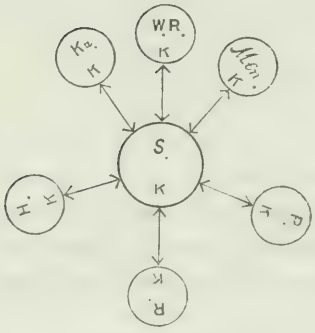
I. Reihe



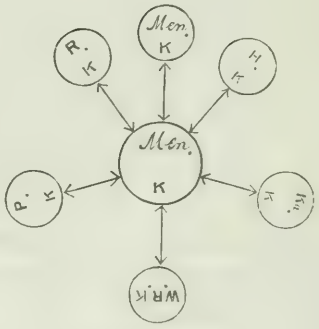
II. Reihe



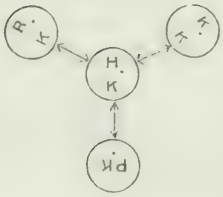
III. Reihe



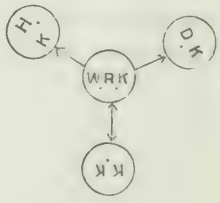
IV. Reihe



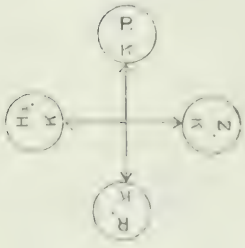
V. Reihe



VI. Reihe



VII. Reihe



3. Kaninchen = M. S.

Schweine = E. S.	Komplementwirkung	ziemlich stark
Pferd = E. S.	„	negativ
Rinder = E. S.	„	„
Menschen = E. S.	„	ziemlich stark
Hunde = E. S.	„	meist negativ od. schr schwach
Ratten = E. S.	„	ziemlich stark
Decumanus = E. S.	„	„ „
Katzen = E. S.	„	meist negativ od. schr schwach
Ziegen = E. S.	Komplementwirkung	meist negativ od. schr schwach
Hammel = E. S.	„	„ „

4. Kaninchen = E. S.

Schweine = M. S.	Komplementwirkung	meist schwach
Pferde = M. S.	„	„ „
Rinder = M. S.	„	„ „
Menschen = M. S.	„	„ „
Hunde = M. S.	„	„ „
Ratten = M. S.	„	„ „
Decumanus = M. S.	„	„ „
Katzen = M. S.	„	„ „
Ziegen = M. S.	„	meist negativ od. schr schwach
Hammel = M. S.	„	„ „

5. Schweine = M. S.

Menschen = E. S.	Komplementwirkung	meist schwach
Pferde = E. S.	„	negativ
Rinder = E. S.	„	„
Hunde = E. S.	„	meist schr schwach od. negativ
Katzen = E. S.	„	„ „
Ratten = E. S.	„	meist ziemlich stark

6. Schweine = E. S.

Menschen = M. S.	Komplementwirkung	meist schwach
Pferde = M. S.	„	ziemlick stark
Rinder = M. S.	„	„ „
Hunde = M. S.	„	„ „
Katzen = M. S.	„	„ „
Ratten = M. S.	„	„ „

7. Menschen = M. S.

Menschen = E. S.	Komplementwirkung	ziemlich stark
Hunde = E. S.	„	schr schwach od. negativ
Katzen = E. S.	„	„ „
Ratten = E. S.	„	meist schwach
Pferde = E. S.	„	negativ
Rinder = E. S.	„	„

8. Menschen = E. S.

Menschen = M. S.	Komplementwirkung	ziemlich stark
Hunde = M. S.	„	meist schwach
Katzen = M. S.	„	„ „
Ratten = M. S.	„	ziemlich stark
Pferde = M. S.	„	„ „
Rinder = M. S.	„	„ „

9. Hunde = M. S.	Katzen = E. S.	Komplementwirkung	....meist negativ
	Pferde = E. S.	,,	....negativ
	Rinder = E. S.	,,	.... ,,
10. Hunde = E. S.	Katzen = M. S.	Komplementwirkung	....meist negativ
	Pferde = M. S.	,,	....sehr schwach
	Rinder = M. S.	,,	.... ,, ,,
11. Weisse Ratten = M. S.	M. Decumanus = E. S...	Komplementwirkung	....ziemlich stark, seiten schwach
	Katzen = E. S.	,,	....meist negativ
	Hunde = E. S.	,,	.... ,, ,,
	Pferder = E. S.	,,	....negativ
	Rinder = E. S.	,,	.... ,,
12. Weisse Ratten = E. S.	M. Decumanus = M. S...	Komplementwirkung	....meist ziemlich stark
	Katzen = M. S.	,,	....ziemlich stark selten schwach
	Hunde = M. S.	,,	.... ,, ,,
	Pferde = M. S.	,,	....meist ziemlich stark
	Rinder = M. S.	,,	....weist ziemlich stark
13. Pferde = M. S.	Meerschweine = E. S. ..	Komplementwirkung	....meist stark
	Kaninchen = E. S.	,,	...sckwach
	Rinder = E. S.	,,	....negativ
	Schweine = E. S.	,,	....meist schwach
	Menschen = E. S.	,,	.... ,, ,,
	Hunde = E. S.	,,	....schr schwach od. negativ
	Ratten = E. S.	,,	....meist schwach
14. Pferde = E. S.	Meerschweinchen = M. S.	Komplementwirkung	....gauf negativ
	Kaninchen = M S.	,,	.... ,, ,,
	Rinder = M. S.	,,	.... ,, ,,
	Schweine = M. S.	,,	.... ,, ,,
	Menschen = M. S.	,,	.... ,, ,,
	Hunde = M. S.	,,	.... ,, ,,
15. Ziegen = M. S.	Meerschweinchen = E. S.	Komplementwirkung	....meist schwach
	Hammel = E. S.	,,	....meist negativ od. gauf schwach
	Kaninchen = E. S.	,,	....schr schwach od. negativ
16. Ziegen = E. S.	Meerschweinchen = M. S.	Komplementwirkung	...meist negativ od. negativ
	Hammel = M. S.	,,	.... ,, ,,
	Kaninchen = M. S.	,,	....meist negativ

Zum Beispiel werden unten nur einige Untersuchungsresultate vorgestellt;

Beispiel 1. Das Bindungsverhältniss zwischen jeden Komplementstücken von den Meerschweinchen u. Schweinen.

(Vergl. Tafel II).

Das Schweine — M. S. verbindet sich mit dem Meerschweinchen = E. S. sehr gut und dadurch ruft eine deutliche Hämolyse hervor. Nämlich wird das

Tafel. II.

No.	Zutaten																			
	5% Z. B. A.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Amb. 0.005	—	—	0.5	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5
	Meerschw. = M.S.	1.5	—	1.5	—	—	—	—	—	1.0	1.5	—	—	—	0.5	1.0	1.5	—	—	—
	Schwein. = E. S.	—	—	—	—	1.5	—	1.5	—	—	—	1.0	1.5	—	0.5	1.0	1.5	—	—	—
	Schwein. = M. S.	—	—	—	—	—	1.5	—	1.5	—	—	1.0	1.5	—	—	—	—	0.5	1.0	1.5
	Meerschw. = E. S.	—	1.5	—	1.5	—	—	—	—	1.0	1.5	—	—	—	—	—	—	0.5	1.0	1.5
	0.85% Nacl-Lös	2.0	2.0	1.5	1.5	2.0	2.0	1.5	1.5	1.0	—	1.0	—	3.5	2.0	1.0	—	2.0	1.0	—
1	Resultat	—	—	—	—	—	—	—	S	###	###	##	###	—	—	S	++	++	###	###
2	,,	—	—	S	—	—	—	—	—	###	###	##	###	—	—	S	+	++	###	###
3	,,	—	—	—	—	—	—	—	—	##	##	++	##	—	—	S	++	—	+	##
4	,,	—	—	—	—	—	—	—	—	##	###	##	###	—	—	—	S	S	##	###
5	,,	—	—	—	—	—	—	—	—	##	##	##	###	—	—	—	—	—	##	###

Z. B. A. = Aufschwemmung d. Ziegenblutkörperchen. Amb. = Amb ozeptor. M. S. = Mittelstück. E. S. = Endstück. Nacl = Kochsalzlösung. ### = Komplette Hämolyse. ## = Fast komplette Hämolyse. ++ = Mittelstarke Hämolyse. + = Schwache Hämolyse. S = Spurweise Hämolyse. — = Hämolyse negativ. —— = Zeichen ohne Zutat.

Schweine=E. S. durch dasselbe vom Meerschweinchen gut vertreten. Im Gegenteil verbindet sich das Meerschweinchen=M. S. mit dem Schweine=E. S. nicht immer und die dabei auftretende Komplementwirkung ist meistens schwach. Jedoch, wenn mann bei solchem Fall die Menge vom Schweine=E. S. etwas mehr zusetzt (210 c.c.), so tritt dann die Hämolyse viel stärker auf. Solche Erscheinung wird nicht nur bei diesem Fall allein, sondern bei den anderen oft beobachtet.

Beispiel 2. Das Bindungsverhältniss zwischen jeden Komplementstücken von den Meerschweinchen u. Menschen.

(Vergl. Tafel III).

Das Menschen=M. S. verbindet sich auch mit dem Meerchweinchen=E. S. sehr gut, und die dadurch erscheinende Komplementwirkung ist fast immer ziemlich deutlich. Das Auftreten der Hämolyse bei diesem Fall ziemlich schnell (15–20 bisweilen 10 M.). Im Gegenteil lässt das Meerschweinchen=M. S. mit dem Menschen=E. S. nicht immer Hämolyse hervorrufen. Wenn auch hierbei die Hämolyse sich hervorruft, ist sie immer schwach. Aber, wenn man bei diesem Fall eine grössere Menge vom Menschen=E. S. braucht, so tritt dann die stärkere Hämolyse auf. Die Geschwindigkeit der Hämolyse ist bei letztem Fall immer langsam (etwa 50–60 M.).

Beispiel 3. Das Bindungsverhälniss zwischen jeden Komplementstücken von den Meerschweinchen u. Ziegen.

(Vergl. Tafel IV).

In dieser Untersuchung musste zunächst ein anderer Ambozeptor Antipferdesblut Kaninchen-Ambozeptor (Titer 1000) vorbereitet werden.

Das Ziegen=M. S. verbindet sich auch mit dem Meerschweinchen=E. S. ziemlich gut. Nämlich wird das Ziegen=E. S. durch dasselbe vom Meerschweinchen gut ersetzt, und das Meerschweinchen=M. S. wird gleichzeitig durch desselbe vom Ziegen vertreten. Im Gegenteil verbindet das Meerschweinchen=M. S. mit dem Ziegen=E. S. sehr schwer. Nur, wenn man eine grössere Menge vom Ziegen=E. S. dabei benutzt, so bemerkt mann eine Schwache Hämolyse. Man bemerkt dass das Mittel- u. Endstück vom Ziegen selbst in seinem Zusammentreffen keine od. ganz schwache Komplementwirkung zeigen; wenn in solchem Fall das Meerschweinchen=E. S. zur Mischung von Ziegen=M. S. u. E. S. zugesetzt wird, so tritt dann stärkere Hämolyse auf. Diese Erscheinung ist in der Weise zu verstehen, dass das Mangel vom Ziegen=E. S. durch das Meerschweinchen=E. S. quantitativ ersetzt werden musste.

Beispiel 4. Das Bindungsverhältniss zwischen jeden Komplementstücken von dem A- und B-Menschen.

(Vergl. Tafel V).

Zum Untersuchungsmaterial sind zunächst Zwei Menschen-Komplemente; d.

Tafel III.

No.	Zutaten																			
	5% Z.E.A.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Amb. (5.005)	—	—	0.5	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5
	Meerschw.=M.S.	1.5	—	1.5	—	—	—	—	—	1.0	1.5	—	—	—	0.5	1.0	1.5	—	—	—
	Mensh.=E.S.	—	—	—	—	—	1.5	1.5	—	—	—	1.0	1.5	—	0.5	1.0	1.5	—	—	—
	Mensh.=M.S.	—	—	—	—	1.5	—	—	1.5	—	—	1.0	1.5	—	—	—	—	0.5	1.0	1.5
	Meerschw.=E.S.	—	1.5	—	1.5	—	—	—	—	1.0	1.5	—	—	—	—	—	—	0.5	1.0	1.5
	0.85% Nacl-Lös.	2.0	2.0	1.5	1.5	2.0	2.0	1.5	1.5	1.0	—	1.0	—	3.5	2.0	1.0	—	2.0	1.0	—
1	Resultat	—	—	—	—	—	—	—	—	###	###	++	###	—	—	—	—	+	##	##
2	„	—	—	—	—	—	—	—	—	++	##	##	##	—	—	—	—	+	##	##
3	„	—	—	—	—	—	—	—	—	###	###	++	##	—	—	—	—	+	##	##
4	„	—	—	—	—	—	—	—	—	++	##	+	++	—	—	—	—	S	+	##
5	„	—	—	—	—	—	—	—	—	++	##	++	##	—	—	—	—	S	++	##

Tafel IV.

No.	Zusätze																			
	5% P.B.A. (0.05)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Ams. (0.05)	—	—	0.5	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5
	Meerschw. = M.S.	1.5	—	1.5	—	—	—	—	—	1.0	1.5	—	—	—	0.5	1.0	1.5	—	—	—
	Ziegen = E.S.	—	—	—	—	1.5	—	1.5	—	—	—	1.0	1.5	—	0.5	1.0	1.5	—	—	—
	Ziegen = M.S.	—	—	—	—	—	1.5	—	1.5	—	—	1.0	1.5	—	—	—	—	0.5	1.0	1.5
	Meerschw. = E.S.	—	1.5	—	1.5	—	—	—	—	1.0	1.5	—	—	—	—	—	—	0.5	1.0	1.5
	0,85% Nacl-Lös.	2.0	2.0	1.5	1.5	2.0	2.0	1.5	1.5	1.0	—	1.0	—	3.5	2.0	1.0	—	2.0	1.0	—
1	Resultat	−	−	−	−	−	−	−	−	++	+++	−	−	−	−	−	−	−	S	++
2	„	−	−	−	−	−	−	−	−	++	+++	S	++	−	−	−	S	−	S	++
3	„	−	−	−	−	−	−	−	−	+++	+++	S	++	−	−	−	+	−	++	+
4	„	−	−	−	−	−	−	−	−	+	++	S	+	−	−	−	−	−	S	++
5	„	−	−	−	−	−	−	−	−	+	+++	−	+	−	−	−	−	−	S	+

P. B. A. = Aufschwemmung d. Pferdeblutkörperchen.

Tafel V.

No:	Zutaten																		
	5% Z. B. A.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Amb. (0.005)	—	—	0.5	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5
	A-Mensch. = M.S.	1.5	—	1.5	—	—	—	—	—	1.0	1.5	—	—	—	0.5	1.0	1.5	—	—	—
	B-Mensch. = E.S.	—	—	—	—	1.5	—	1.5	—	—	—	1.0	1.5	—	0.5	1.0	1.5	—	—	—
	B-Mensch = M.S.	—	—	—	—	—	1.5	—	1.5	—	—	1.0	1.5	—	—	—	—	0.5	1.0	1.5
	A-Mensch. = E.S.	—	1.5	—	1.5	—	—	—	—	1.0	1.5	—	—	—	—	—	—	0.5	1.0	1.5
	0.85% Nacl-Lös.	2.0	2.0	1.5	1.5	2.0	2.0	1.5	1.5	1.0	—	1.0	—	3.5	2.0	1.0	—	2.0	1.0	—
1	Resultat	—	—	—	S	—	—	—	—	++	+++	++	+++	—	S	S	++	—	+	+++
2	„	—	—	—	—	S	—	S	—	+	+++	++	+++	—	—	—	++	—	+	+++
3	„	—	—	—	S	—	—	—	—	—	S	+	+++	—	—	+	+++	—	—	+
4	„	—	—	—	—	—	—	—	—	—	++	S	++	—	—	—	S	—	—	+
5	„	—	—	—	—	—	—	—	—	+	+++	+	+++	—	—	—	++	—	S	++

h. das A-Menschen = Komplement (Titer 0.05), u. das B-Menschen = Komplement (Titer auch 0.05), ausgewährt worden. Die zwei Komplementen mussten dann auch in zwei Komponenten, d. h. das Mittelstück u. Endstück zergliedert werden.

Das Mittelstück vom A-Mensch verbindet sich mit dem Endstück vom B-Mensch sehr gut, so dass dadurch eine deutliche Komplementwirkung sich hervorruft. Auch das Mittelstück vom B-Mensch verbindet sich mit dem Endstück vom A-Mensch ebenfalls gut. Man bemerkt manchmal Fall, welcher durch die Bindung des Mittel u. Endstück vom B-Mensch selbst nur schwache Komplementwirkung zeigt. In solchem Fall, wenn man an Statt des Endstück vom B-Mensch dasselbe vom A-Mensch anwendet, so tritt dann hierbei eine stärkere Hämolyse auf. Diese Erscheinung ist in der Weise zu verstehen, dass das Mangel des Endstück vom B-Mensch durch dasselbe vom A-Mensch quantitativ ersetzt sein musste.

Die hämolytische Geschwindigkeit bei der Bindung zwischen jeden Komplementstücken vom A- u. B-Mensch, ist fast immer gleich aufgetreten, wenn das Komplementgehalt vom A- u. B-Mensch in gleicher Grösse ist.

3. Die Ersatzbarkeit zwischen jeden Komplementstücken der Warmblütern und Geflügeln.

In diesem Kapitel möchte ich daran reden, ob es zwischen der Komplementen von den Warmblütern und Geflügeln ein gemeinsames Element gibt od. nicht? Im anderen Worte ausgedruckt, ob ein Komplementstück (Mittel- od. Endstück) des Vogels durch ein Stück (Mittel od. Endstück) des Warmblüters vertreten werden kann od. nicht?

Wie im Eingang dieser Arbeit ausdrücklich gesagt ist, wird das mit Ziegenblutkörperchen vorbehandelte inaktivierte Serum durch die Einwirkung von frischen Sera der Meerschweinchen, Hühnern, Enten, etc., wieder reaktiviert. Daraus könnte man vielleicht sagen, dass die Komplemente aus den Warmblütern und Vögeln als eine gleiche Substanz betrachtet werden können. Um diese Vermutung wirklich zu machen, habe ich die folgenden Experimente angestellt.

Als die Untersuchungsmaterialien wurden die Komplemente von den Meerschweinchen, Menschen, Hühnern, Wildenten etc. ausgewählt. Die Untersuchungsmethode ist ganz gleich mit derjenige, welche schon in dem III. Kapitel eingehend beschrieben ist. Zunächt musste das Komplement der oben erwähnten Tieren und Vögel auch in zwei Komponente zergliedert werden. Dann liessen teils dem Vogel-mittelstucke das Tier-Endstück und teils dem Tier-Mittelstücke des Vogel-Endstück in gekreuzter Weise einwirken. Hierbei sind auch alle Kontrollversuche gar nicht vernachlässigt worden.

Diese Untersuchungen fielen immer bedeutend schwieriger auf, als diejenige, welche schon in dem III Kapitel ausgeführt worden sind, sodass ich hierbei bei den über 20 maligen Experimenten schliesslich nur einige sichere Resultate eruirte. Hier will ich nur einen Untersuchungergebniss zum Beispiel vorstellen.

Beispiel 5. Das Bindungsverhältniss zwischen jeden Komplementstücken von den Meerschweinchen u. Enten.

(Vergl. Tafel VI).

Bevor ich dieses Verhältniss klar zu machen, musste ich zunächst das Komplementgehalt der Enten bestimmen. Unter 8 Untersuchungsfällen schwankt sich das Enten-Komplement zwischen 0.05–0.06; Zum Experimente wurde immer diejeniges Komplement angewendet, welches das höchsten Titer besitzt.

Wie es in dem VI Tafel veranschaulich ist, verbindet sich das Entenmittelstück mit dem Meerschweinchen-Endstück fast immer ziemlich gut und die dadurch hervorgerufene Hämolyse tritt sich ziemlich deutlich auf (Hämolytische Geschwindigkeit etwa 30 M.). Mit einem anderen worte ausgedrückt, wird das Enten-Endstück durch das Meerschweinchen-Endstück und gleichzeitig das Meerschweinchen-Mittelstück durch das Enten-Mittelstück gut vertreten. Bemerkenswert ist es, dass die hämolytische Stärke, welche durch die Bindung des Enten-M.S. mit dem Meerschweinchen = E. S. hervorgerufen wird, viel stärker ist, als diejenige, welche durch die Bindung des Enten = M. S. mit seinem eigenen Endstück sich erscheint. Schliesslich verbindet sich das Meerscheinchen = M. S. mit dem Enten = E. S. gar nicht, so dass man dabei keine Hämolyse beobachten konnte.

Im Allgemeinen wird das Vogel-Endstück durch das Meerschweinchen oder Menschen-Endstück etc. ziemlich gut ersetzt. Mit einem anderen Worte ausgedruckt, erkennt man dabei, dass durch die Bindung vom Vogel-mittelstück mit dem Tier-Endstück eine Komplementwirkung sich hervorruft. Einzelne Resultate werden auch unten kurz zusammengefasst;

Hühner = M. S. mit Meerschweinchen = E. S. — Stärke d. Hämolys. — Schwach od. negativ.
Wild-enten = M.S. mit Meerschweinchen = E. S. — Stärke d. Hämolys. — Ziemlich stark od. schwach.
Wild-enten = M. S. mit Menschen = E. S. — Stärke d. Hämolys. — Schwach.
Meerschweinchen = M. S. mit Hühner = E. S. — Stärke d. Hämolys. — negativ.
Meerschweinchen = M. S. mit Wild-enten = E. S. — Stärke d. Hämolys. — negativ.
Menschen = M. S. mit Wild-enten = E. S. — Stärke d. Hämolys. — negativ.

Wie es in der oben angegebenen Zusammenfassungen übersichtlich ist, ist die Hämolyse am stärksten, welche durch die Bindung vom Wild-enten-Mittelstück mit dem Meerschweinchen-Endstück hervorgerufen wird und das Mittelstück von den Tieren mit dem Vogel-Endstück lässt sich nie Hämolyse vervorrufen.

Mit dem anderen Worte ausgedruckt, erkennt man, dass das „ Albuminstück " von den Warmblütern durch dasselbe von den Vögeln gut ersetzt und das „ Globulinstück " von den Vögeln durch dasselbe von den Warmblütern auch gut ersetzt werden können. Trotzdem kann das „ Globulinstück " von den Warmblütern durch dasselbe von den Vögeln nie vertreten werden.

Noch kürzer ausgedruckt, zwischen jeder Komplementstücken von den Warmblütern und Vögeln kann nur eine „ partielle Ersatzbarkeit " bestätigt werden.

Tafel VI.

No:	Zutaten																		
	5% Z. B. A.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Amb. 10.000×	—	—	0.5	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5
	Enten = M.S.	2.0	—	2.0	—	—	—	—	—	1.0	2.0	—	—	—	1.0	1.5	2.0	—	—	—
	Meerschw. = E.S.	—	—	—	—	2.0	—	2.0	—	—	—	1.0	2.0	—	1.0	1.5	2.0	—	—	—
	Meerschw. = M.S.	—	—	—	—	—	2.0	—	2.0	—	—	1.0	2.0	—	—	—	—	1.0	1.5	2.0
	Enten = E.S.	—	2.0	—	2.0	—	—	—	—	1.0	2.0	—	—	—	—	—	—	1.0	1.5	2.0
	0.85% Nacl-Lös.	2.5	2.5	2.0	2.0	2.5	2.5	2.0	2.0	2.0	—	2.0	—	4.5	2.0	1.0	—	2.0	1.0	—
1	Resultat	—	—	—	—	—	—	—	—	—	+	++	++	—	S	++	++	—	—	—
2	„	—	—	—	S	—	—	—	—	—	+	++	++	—	S	++	++	—	—	S
3	„	—	—	—	S	—	—	—	—	S	++	++	++	—	—	+	++	—	—	S
4	„	—	—	—	—	—	—	—	—	—	S	++	++	—	—	—	+	—	—	—
5	„	—	—	—	—	—	—	—	—	—	+	++	++	—	—	S	++	—	—	—

Zusammenfassende Betrachtungen.

Sehen wir die oben angestellten Experimente noch einmal durch, so können wir wohl verstehen, dass die jeden Komponente von den verschiedenen Warmblüterkomplementen miteinander Erstazbarkeit darstellen können. Im anderen Worte, wird das Endstück vom B-Tier durch dasselbe vom A-Tier ersetzt, d. h. das Mittelstück vom B-Tier kann mit dem Endstück vom A-Tier ein Komplement bilden. In gleicher weise stellt das Mittelstück vom A-Tier mit dem demselbe vom B-Tier auch eine Komplementwirkung dar. Hierbei finden sich aber die Fälle, dass das Mittelstück vom B-Tier mit dem Endstück vom A-Tier deutliche Hämolyse hervorruft, während das Mittelstück vom A-Tier mit demselbe vom B-Tier nur ganz schwache Komplementwirkung zeigt oder gar keine Hämolyse dadurch sich hervortritt. Solche Ergebnisse können meiner Ansicht nach vielleicht von den folgenden Gründen verursacht sein;

(1) Bezieht es sich von der Menge jeden Komplementstücke? Zur Erklärung dieser Verhältnisse werde ich hier mit dem Beispiel andeuten. Beobachtet man die Ersatzbarkeit jeder Komplementstücke zwischen den Meerschweinchen und Kaninchen, so erkennt man wohl darauf, dass das Kaninchen-Mittelstück mit dem Endstück von Meerschweinchen eine deutliche Hämolyse zeigt, während das Meerschweinchen-Mittelstück mit dem Kaninchen-Endstück nur eine schwache Komplementwirkung darstellt. Wenn man in solchem Fall, eine noch gössere Menge von dem Kaninchen-Endstück einwirken lässt, so steigt die hämolytische Fähigkeit deutlich auf. Derartige Tatsache wird natürlich oft bei den weit anderen Tierarten bemerkt. Woraus bezieht es sich eigentlich? Wie es schon von den mehreren Autoren und von mir selbst anerkannt ist, ist die Menge jeder Komplementstücke von den Tieren ziemlich verschieden. In einem Tiere ist viel Endstück enthalten, während dasselbe in einem anderen Tier sehr spärlich oder gar nicht nachgewiesen wird. Trotzdem schwankt sich die Menge vom Mittelstück nach der Verschiedenheit von den Tierarten nicht so erheblich. Von diesem Grunde, ist es wohl verständlich, warum das Mittelstück vom B-Tier mit dem Endstück vom A-Tier deutliche Hämolyse hervorruft während das Mittelstück vom A-Tier mit dem vom B-Tier schwache oder gar keine Komplementwirkung zeigt. Anders gesagt, müsste solches Verhältnisse von dem quantitativen Unterschied jeder Endstüke bei den Tieren verursacht sein. Hierzu wird noch ein besserer Beispiel vorgestellt;

Lässt man dem Pferde- oder Rinder-Mittelstück eine gewisse Menge vom Endstück der Meerschweinchen, Kaninchen, Menschen, Schweine, Ratten, Hunden, Katzen, etc. einwirken, so kann man recht verschiedene stärke der Komplementfähigkeit ersehen. Diese Verhältnisse müssen wohl nach der Verschiedenheit der im solchen Tierkomplementen erhaltenden Endstück-Menge erklärt werden. Ferner stelle ich noch einen folgeden Fall vor.

Lässt das Mittelstück vom Kaninchen mit seinem eigenen Endstück sich verbinden, so tritt hier ganz schwache Hämolyse auf, oder gar nicht. Lässt man

hierzu noch eine kleine Menge von Meerschweinchenendstück einwirken, so tritt dann eine stärkere Hämolyse auf. Dieses Verhältniss wurde nicht nur zwischen den Meerschweinchen und Kaninchen, sondern auch zwischen den ersteren und den viel anderen Tierenarten beobachtet. Diese Tatsache ist davon zu begreifen, dass die Spärlichkeit des Endstücks von Kaninchen und von den anderen Tieren durch das Meerschweinchen-Endstück quantitativ ersetzt ist.

(2) Handelt es sich um die Affinität zwischen jeden Komplementstücke? Zum Beispiel wird hier das Verhältniss zwischen den Menschen- und Ratten-Komplementstücken erwähnt. Vorausgesetzt, dass es hier das Menschenkomplement (Titer; 0.05) und das Rattenkomplement (Titer; auch 0.05) gibt. Diese zwei Komplemente werden nach gleicher Methode ins Mittel- und Endstuck gespaltet. Das Menschen-Mittel- und Endstück (je 1.5 : 1.5) zeigten fast komplette Hämolyse (##). Auch beim Rattenkomplemente zeigten sein Mittelstück 1.5 mit seinem eigenen Endstück 1.5 auch fast komplette Hämolyse (##). Vom diesem Grunde aus mussten das Ratten-mittelstück 1.5 mit dem Menschen-Endstück 1.5 und ebenfalls das Menschen-Mittelstück 1.5 mit dem Ratten-Endstuck 1.5 logisch eine gleichstarke Komplementfähigkeit darstellen. Jedoch ist die durch die Bindung von den ursprunglich verschiedenen zwei Komponenten auftretende Hämolyse viel schwächer als dieselbe durch die Vereinigung vom Menschen-Mittelstück mit seinem eigenen Endstück (1.5 : 1.5) und durch die Bindung vom Ratten-Mittelstücke mit seinem eigenen Endstück (1.5 : 1.5) hervorgerufen wird. Diese Verhältnisse können nicht nur nach der quantitativen Verschiedenheit der jeden Komplementstücke erklärt werden, sondern auch musste die Affinität zwischen jeden Stücken gedacht werden.

Als einen noch besseren Beispiel wird auch der folgende Fall vorgestellt;

Das Komplement Titer vom Verfasser ist 0.05 und derselbe von einem Mädchen (Saito, 20 Jahre alt) auch 0.05. Das Mittelstück vom Verfasser, 1.5 c.c. mit das Endstück 1.5 c.c. von Saito und das Endstück vom Verfasser 1.5 c.c. mit dem Endstück 1.5 c.c. von Saito stellten immer gleichstarke Komplementwirkung dar (komplette Hämolyse). Auf Grund der oben vorgestellten zwei Tatsachen glaube ich wohl schliessen zu können, dass die hämolytische Stärke, welche durch die Bindung jeden Komplementstücke vom A- und B-Tier hervorgerufen wird, nicht nur es sich von der Menge jeder Stücke handelt, sondern auch ihre gegenseitige Affinitätstärke dabei eine wichtige Rolle zu spielen ist. Allerdings ist diese Affinität meiner Ansicht nach viel stärker zwischen den Komponenten aus homologener Rasse, als aus heterologener.

(3) Wie das sog. „dritte Komponent" von Ritz sich dabei handelt? Um die oben erwähnten Verhältnisse noch klarer zu machen, muss hierbei noch das dritte Komponent in Frage gestellt werden. Jedoch möchte ich heute davon nicht viel reden, sondern hoffe ich in einer späten Gelegenheit darüber zu reden, indem ich unten nur ein Worte dazu brauche, dass die Bedeutung des dritten Komponentes zur Hämolyse nie vernachlässigt werden kann.

Es wird hier auch mit einem Beispiel erklärt. Lässt man zum Menschenmittelstück das Hunde-Endstück einwirken, so tritt keine Hämolyse auf. Wenn in solchem Fall das Hunde-Endstück noch mehr hinzugesetzt wird, so bemerkt man trotzdem kein Auftreten von Hämolyse. Endlich, fügt man dieser obigen Mischung vom Menschen-Mittelstück und Hunde-Endstück, eine gewisse Menge vom III Komponent hinzu, so tritt dann die erkennbare Hämolyse auf. Solche Erscheinung wird nicht nur im Fall bei den Menschen- und Hunde-Komplementen allein, sondern auch zwischen den Komplementstücken von den anderen verschiedenen Tierarten beobacht. Ich meine daher, dass das III Komponent von Ritz für die Ersatzbarkeit zwischen jeden Komplementstücken eine nicht vernachlässigte Bedeutung haben muss.

(4) Das zeitliche Verhalniss zum Auftreten der Hämolyse. Über das zeitliche Verhaltniss des Auftreten der Hämolyse, welche durch die Bindung des Mittelstücks vom B-Tiers mit dem Endstück vom A-Tier hervorgerufen wird, werde ich hier nicht davon näheres reden, sondern bleibe ich nur ein paar Worte dazu zu benutzen. Ersieht man die Geschwindigkeit der Hämolyse bei der Bindung von den Meerschweinchen- und Kaninschenkomplementstücken, so erkennt man daraus, dass die Hämolyse, welche durch die Bindung des Mittel- und Endstücks vom Meerschweinchen selbst sich hervorruft, am allerschnellsten auftritt, und dann die Hämolyse, welche durch die Bindung vom Kaninchen-mittelstück und Meerschweinchen-Endstück sich hervorruft, zweitens auftritt.

Also wird es im Allgemeimen darauf auf gefasst, dass die Hämolyse, welche die Bindung des Mittelstücks von einem Tier (wenn das grössere Komplementgehalt besitzt als die anderen) mit seinem eigenen Endstück hervorgerufen wird, schneller auftritt, als die Hämolyse, welche durch die Bindung des Mittelstücks von einem anderen Tiere (welches ein geringeres Komplement enthält) mit seinem eigenen Endstück veranlasst wird. Wenn das B-Tier, geringeres Komplement enhält, so tritt die Hämolyse, welche durch die Bindung des Mittel- u. Endstück vom B-Tier selbst hervorgernfen wird, sehr spät auf. In solchem Fall, wenn mann an Statt des Endstück vom B-Tier, dasselbe vom A-Tier, welches ein grösseres Komplement besitzt, als B-Tier, benutzt, so tritt dann die Hämolyse im Allgemeinen schneller auf.

III. Schlusssätze.

Fassen wir die oben angestellten Experimente noch ein Mal kurz zusammen, so können wir zum folgenden Schluss kommen;

(1) Die Komplemente, welche sich weit in der Welt der verschiedenen Lebwesen vorhanden, können wohl als eine gleiche Substanz betrachtet werden.

(2) Der Grund, warum solche Komplemente von gleicher Natur sind, wird durch die Ersatzbarkeit zwischen jeden Komplementstücken von den verschieden Lebwesen, beantwortet.

(3) Diese Ersatzbarkeit wird nicht nur zwischen den komplementstücken von den Warmblütern allein, sondern auch zwischen denen von den Warmblüter- und der Geflügel-Komplementen nachgewiesen.

(4) Zwischen jeden Komplementstücken von den Warmblütern stellt im Allgemeinen eine „totale Ersatzbarkeit" dar, während zwischen denen von den Warmblütern und Geflügeln nur eine „partielle Ersatzbarkeit" bestätigt werden kann.

(5) Solche Ersatzbarkeit scheint wohl entweder nach dem quantitativen Verhältnisse zwischen jeden Komplementstücken oder nach der Affinitätsstärke zwischen ihnen verschieden zu sein.

(6) Das sog. III. Komponent kann bei solcher Ersatzbarkeit nie vernachlässigt werden.

Zum Schluss ist es mir eine angenehme Pflicht, Herrn Direktor, Prof. M. Nagayo für die liebenswürdige Unterstützung dieser Arbeit, und Meinem hoch verehrten Lehrer, Herrn Prof. K. Ishiwara für seine freundlichste Leitung zur Arbeit, meinen herzlichsten Dank auszusprechen.

Gleichzeitig drücke ich noch Herrn Dr. T. Otawara für das freundliche Hilfe zur Arbeit meinen warmsten Dank aus.

Diese Arbeit ist noch nicht ganz fertig und der übrige unvollendete Teil ist gerade auf dem Wege der Untersuchung. Deszember 1921. Tokyo,

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La plej simpla metodo por serodiagnozo de sifiliso.

(la unua raporto)

De **M. Murata** (村田正太).

El la laboratorio de la sesa sekcio por bakteriologio kaj serologio
(Sekci-ĉefo. prof. Tenji Taniguĉi)

Organekstrakto ĥolesterinita estis uzata kiel antigeno ĉe Wassermann-reakcio unuafoje je la jaro 1911 de Sachs, kaj pli malfrue Sachs kaj Georgi ankaŭ elektis la saman reakciilon ĉe sia precipitatreakcio. Tiamaniere organekstrakto ĥolesterinita estis konata al ni kiel reakciilo de sifilisa serodiagnozo jam de plimulte ol dek jaroj. Sed nur kontentiĝante uzi la fakton, ke ĥolesterino pliakrigas la sentemecon de organekstrakto, oni neniam funde pripensis, kia interrilato ekzistas inter organekstrakto kaj ĥolesterino.

Por klarigi tiun ĉi interrilaton, unue mi miksis salsolvaĵon fiziologian 1 kc. kun 0·01 kc. da 1% alkohola ĥolesterinsolvaĵo, kaj mi rimarkis tuj aperantan precipitaton. Sed se mi nun miksis salsolvaĵon 1 kc. kun 0·01 kc. da 1% alkohola ĥolesterinsolvaĵo antaŭe miksita kun 0.1 kc. da organekstrakto, nenia precipitato aperis krom tio, ke la fluidaĵo iomete opaliĝis. Tiu ĉi eksperimento instruis min, ke organekstrakto devas esti protektkoloido por ĥolesterino, kiu sola estas tro sentema kiel reakciilo, kaj ke oni povas alĝustigi la proporcion inter ĥolestirino kaj organekstrakto, por ke oni ricevu taŭgan reakciilon.

Unue, en tri provtubojn, en kiujn mi antaŭe enmetis respektive 0·01, 0·02, 0·03 kc. da ĥolesterinsolvaĵo, mi enversis po 1 kc. da salsolvaĵo. La provtubo kun 0·01 kc. donis al mi la deziritan kvanton da precipitato, dum en la aliaj sin montris tro multe da precipitato. Due, enmetinte en 5 tubojn po 0·01 kc. da ĥolesterinsolvaĵo, mi enmiksis respektive 0·5, 0·4, 0·3, 0·2, 0·1 kc. da ekstrakto, antaŭ ol rapide enversi 1 kc. da salsolvaĵo. Lasinte ilin stari 20 horojn en termostato je 37°C, mi trovis, ke en la tuboj enhavantaj pligrandajn kvantojn ol 0·2 kc. la miksaĵoj montris tiel tro densan blankecon, ke ĝi malhelpus la prijuĝon de precipitato. Mi do plu ekzamenis kun kvantoj malpli ol 0·2 kc. da ekstrakto kiel jene : 0·18, 0·16, 0·14, 0·12, 0·1, 0·08, 0·06, 0·04, 0·02 kc.. La tubo enhavanta 0·18 kc. estas ankoraŭ tro dense blanka, dum la tuboj kun 0·06, 0·04 kaj 0·02 kc. donis precipitaton. El tiu ĉi eksperimento mi eksciis, ke la konvena kvanto de la organekstrakto devas esti inter 0·16 kaj 0·06 kc. por 0·01 kc. da ĥolesterinsolvaĵo. Ju pli multe la dozo alproksimiĝas al 0·16 kc., des malpli senteman reakciilon oni ricevas, dum ju pli multe la dozo alproksimiĝas al 0·06 kc., des pli senteman reakciilon oni havas, ĉar la firmeco de reakciilo devas dependi de la

dozo de ĝia protektkoloido, t.e. organekstrakto. Por doni al la reakciilo la plej grandan firmecon kaj samtempe la plej fortan sentemecon kiel eble, mi prove elektis 0·1 kc. da ekstrakto por 0·01 kc. da ĥolesterinsolvaĵo. Antaŭe kunmiksinte 0·1 kc. da ĥolesterinsolvaĵo kun 1 kc. da ekstrakto, mi rapide enversis 10 kc. da salsolvaĵo fiziologia en la miksaĵon. Mi surtavoligis tium reakciilon sur diversajn serojn inaktivajn jam provitajn laŭ Wassermann-reakcio, kaj trovis, ke la plej forte pozitivaj (###) seroj tuj montris, la mezforte pozitivaj (##) iom pli malfrue, kaj la malforte pozitivaj (+) ankoraŭ pli malfrue, blankan ringon, dum la negativaj (—) montris nenian malklaraĵon sur la tuŝebenaĵo inter la reakciilo kaj la sero. La grado de dikeco de ringo dependis de la forteco de pozitiveco. La finiĝon de la reakcio oni decidas laŭ la ekapero de ringo blanka ĉe la plej malforte pozitiva sero uzata kiel kontrolo.

Se la reakciilo tiamaniere preparita, ekzemple, ne reakcias kum + sero, dum ### kaj ## seroj montras blankan ringon, oni povas plifortigi la sentemecon de la reakciilo malmultigante la ekstrakton, kontraŭe, se ankaŭ negativa sero montras blankan ringon, oni povas malpliakrigi la sentemecon de la reakciilo plimultigante la dozon de la ekstrakto.

Mia ekstrakto estas preparita jenmaniere: de la koro de bovo konservita 24 horojn en glaciejo mi deprenis muskolon, tranĉis ĝin en malgrandajn pecetojn kaj muelis ilin kune kun vitra pulvoro. Por 1 grm de la muskolo muelita mi aldonis 5 kc. da 95% alkoholo. La ekstrakto lasita stari ĉirkaŭ 48 horojn en laboratoria temperaturo estas filtrita per filtra papero; la filtraĵo estas refiltrita post 24 horoj. Aldoninte al tiu filtraĵo duoblan kvanton da 95% alkoholo, mi ricevis mian organekstrakton.

Se oni tiamaniere havas ekstrakton, oni mezuru antaŭ ĉio ĝian dozon konvenan por 0·01 kc. da 1% alkohola ĥolesterinsolvaĵo. Oni povas miksi la ambaŭ fluidaĵojn en la trovita proporcio kaj konservi tiun ĉi diagnozilon en bruna boteleto.

Ĝenerale oni ricevas maldense koloran solvaĵon de koloido ĉe rapida miksado kaj plidense malklariĝantan ĉe plimalrapida miksado. La pli maldensa solvaĵo havas pli grandan firmecon kaj malpli fortan sentemecon, dum la pli densa havas malpli grandan firmecon sed plifortan sentemecon. Tial oni devas salsolvaĵon enversi ĉiam kun definita rapideco; tio estas tre grava. Se en balonon de Erlenmeyer (200 kc.) kun 11 kc. de mia diagnozilo oni enversas kiel eble plej rapide 100 kc. da salsolvaĵo per mezurcilindro de 100 kc., oni ricevas la reakciilon kun kriteria opaleco dezirata de mi.

La reakciilon oni uzu de 20 minutoj ĝis 5–6 horoj post la miksado. Povas esti, ke tro freŝa reakciilo posedas tiel malfortan sentemecon, ke ĝi ne reakcias kun malforte pozitiva sero, dum ke tro malfreŝa reakciilo reakcias eĉ kun negativa sero, ĉar la sentemeco de la reakciilo plifortiĝas kun la tempo.

Estas rekomendinde, ke oni komencu kontroli la reakcion per ###, ##, +, ± kaj — seroj. Sed spertulo povas elekti nur + kaj — serojn kiel kontrolojn.

Sero devas esti protektata kontraŭ hemolizo, ĉar sero hemolizinta povas montri sub la tuŝebenajo blanketan ringon, kiu povas malfaciligi la prijuĝon de rezultato. Se seroj estas nur bonaj, oni povas, komparante kun la kontroloj, facile juĝi, ĉu ili estas pozitivaj (kaj kiaj pozitivaj) aŭ negativaj.

Se oni havas malbonajn serojn oni povas apogi sin sur mian alian metodon: oni enmetu tiuokaze respektive 0·1, 0·15, 0·2, 0·25, 0·3 kc. de la sero ekzamenota en tubojn kaj aldonu po 1 kc. de la reakciilo en ĉiun tubon. Elpreninte post 20 horoj la tubojn el termostato je 37°C, oni serĉu la tubojn, en kiuj la kontrolaj seroj donas precipitaton. Tiel kontrolante oni facile povas juĝi la rezultaton. Ĉe la uzado de mia nuna reakciilo, tiu sero, kiu povas doni precipitaton je 0·1 kc, estas ###, kaj tiu donanta je 0·15 kc. estas ##, kaj tiu sero, kiu donas je 0·25 aŭ 0·3 kc., estas +. Negativa sero ne donas precipitaton eĉ je 0·3 kc. Por eltrovi la precipitaton ĉe tiu metodo oni uzu lupeon.

Ekzameninte 62 serojn laŭ mia metodo kaj samtempe laŭ Wassermann-reakcio mi trovis, ke la rezultato de mia metodo en ĉiuj okazoj koincidis kun tiu de Wassermann-reakcio (pozitivaj 16, negativaj 45), esceptante nur unu okazon, en kiu la rezultato de mia metodo estis —, dum la Wassermann-reakcio montris ±. Sed se oni pripensas, ke la diferenco inter ± kaj — havas en si tre malgrandan signifon, oni povas eble diri, ke la rezultato de mia ringprovo koincidas kun tiu de Wassermann-reakcio laŭ la metodo de Browning ĝis ĉirkaŭ 100%.

Kvankam la nombro de la seroj ekzamenitaj de mi ankoraŭ ne estas sufiĉe multa, ŝajnis al mi, ke mia metodo neniel estas malpli bona ol la plej bonaj metodoj publikigitaj ĝis nun.

Krom tio mia metodo havas jenajn plibonajn punktojn ol la jam publikigitaj metodoj por serodiagnozo de sifiliso: 1. Uzante mian metodon oni povas facile ricevi konvene senteman reakciilon nur regulante la dozon de la organekstrakto, kiu estas necesa por 0·01 kc. da 1% ĥolesterinsolvaĵo. 2. Ĉiu povas prepari ĉiam tiun reakciilon, kiu havas definitan sentemecon. 3. Manipulado estas tre simpla. 4. La rezultato estas ne dusenca kaj ĉiu povas facile juĝi la rezultaton. 5. Elspezo estas tre malgranda. 6. Laŭ mia metodo forte pozitivaj seroj reakcias tuj post la surtavoligo, eĉ tre malforte pozitivaj seroj ĝenerale ne postulante kvaronhoron, dum laŭ la aliaj ekzistantaj metodoj oni bezonas rimarkinde pli multon da tempo. 7. Ĉe la aliaj metodoj termostato estas nepre bezona, sekve, la plej multe da kuracistoj ne estas kapablaj ekzameni serojn, kontraŭe, mia metodo permesas la serodiagnozon al ĉiu kuracisto, kiu ne havas specialan laboratorion, se li nur havas la diagnoziilon preparitan. (*publikigita en Japanische Zeitschrift für Dermatologie u. Urologie. Vol. 22, N-ro 11, Nov. 1922*).

La plej simpla metodo por serodiagnozo de sifiliso.

(la dua raporto)

De **Murata Masataka** (村田正太).

Gis hodiaŭ (la 23^an de Decembro 1922) mi ekzamenis laŭ mia metodo 303 serojn, kiuj samaj estis ankaŭ ekzamenitaj de miaj afablaj kolegoj S-roj Nagajama kaj Suzuki per la Wassermann-reakcio laŭ la metodo de Browning (en Glasgovo) sub la kontrolo de prof. T. Taniguĉi. Jen la rezultato:

Tabelo I

rezultato de		nombro de seroj
la Wassermann-reakcio	mia reakcio	
###	###	37
	##	9
##	##	7
	###	1
	+	2
+	+	14
	##	3
−	−	198
	+	2
	##	3
±	±	2
	+	3
	−	22
sumo		303

Nun, komparante miajn rezultajn kun tiuj de la Wassermannreakcio pri 276 seroj, kies diagnozo estas sendube pozitiva aŭ negativa, ni ricevas jenajn ciferojn:

Tabelo II

rezultato de		nombro de seroj
la Wassermann-reakcio	mia reakcio	
pozitiva	pozitiva	73
	negativa	0
negativa	negativa	198
	pozitiva	5
sumo		276

El la 5 nekoincidaj seroj mi povis certigi al mi pri la tri, ke la unua venis de pulmokarcinomulo, la dua de ftizulo morte suferanta kaj la tria de tiu, kiu suferis de pleŭrito eksuda kaj antaŭ kelke da monatoj ricevis injekton de Salvarsano pro sifiliso. Pri la du ceteraj seroj bedaŭrinde ne prosperis al mi informiĝi iliajn anamnezojn.

Se oni atente esploras ĉi-suprajn tabelojn, sin turnante al la anamnezoj koncernantaj, oni konvinkos sin, kiel trafe koincidas mia rezultato kun tiu de la Wassermann-reakcio. (*publikigita en Japanische Zeitschrift für Dermatologie u. Urologie. Vol. 23, N-ro 2, Febr. 1923*).

Beiträge zu Serologischen Untersuchungen über das Enteral und Parenteral eingeführte, Artfremde Eiweiss.

Von

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Inhalt.

1. Einleitung.
2. Versuchsmethoden.
3. Über die Präzipitation des pflanzlichen Eiweisses.
4. Geht das enteral eingeführte Eiweiss durch die Wand des Verdauungskanals durch?
5. Geht das Eiweiss durch die Wand des Verdauungstraktus der Tiere unter Normalfütterung durch?
6. Über die antigenösen Veränderungen artfremden Eiweisses in der Leber.
7. Über die Veränderungen der Kohlenhydrate in der Leber.
8. Schlussbetrachtungen.

Literatur.

Tabellen.

I. Einleitung.

Heutiger Physiologie nach gehen die von der Darmwand resorbierten Nahrungsstoffe durch zwei Gänge ins Blut über: die Pfortader und die Chylusgefässe. Es sind überhaupt Eiweiss, Kohlenhydrate, Salze, Flüssigkeiten, geringfügiges Fett, die den ersteren Gang gehen. Sie treten durch Epithelzellenschicht der Darmzotten, Kapillargefässe, Mesenterialgefässe und Pfortader in die Leber ein. Und es ist ausschliesslich das Fett, welches den letzteren geht. Dieses tritt auch durch Epithelzellenschicht der Darmzotten, Chylusgefässe, Lymphgefässe in Mesenterium und Ductus thoracicus ins Blut ein. Und während manche Autoren genaue

serologische Untersuchungen über biologische Reaktion parenteral eingeführten Eiweisses versucht haben, bleibt enteral eingeführtes Eiweiss, das in dem Stoffwechselsvorgange von grösster Wichtigkeit ist, noch nicht genug erforscht. Dass man den Versuchstieren artfremdes Eiweiss enteral gibt und dessen Schicksal vom serologischen Standpunkt aus studiert, um diese Frage zu lösen, ist im Stoffwechselsvorgang von grosser Bedeutung und trägt sehr zum Studium enteraler Immunisierung bei. Nach meiner Meinung wollte ich unter Anleitung von Herrn Prof. K. Ishiwara die Veränderungen des Eiweisses unter Anwendung der Präzipitation und Anaphylaxie experimental studieren und zugleich die Funktion der Leber auf Kohlenhydrate quantitativ untersuchen.

Bevor ich jetzt die Resultate meiner Untersuchungen erwähne, will ich zuerst die Ergebnisse der Versuche von Autoren über die Präzipitation und Anaphylaxie gegen enteral eingeführtes Eiweiss kurz darstellen.

Hamburger und Sperk[16] gaben an, das in Begleitung der Speise eingeführte artfremde Eiweiss ist nicht nachweisbar im Blute des Tieres durch Präzipitation. Im allgemeinen wird artfremdes Eiweiss bei dessen Verdauung zu Atomkomplex zerstört, d.h. es wird infolge der Wirkungen der Salzsäure und des Fermentes, welche beide von Magendarmschleimhaut abgesondert werden, in Albumose, Pepton und Aminosäure stufenweise analysiert, resorbiert und endlich infolge der abermaligen Wirkung des Fermentes dem Eiweisse assimiliert, dessen Struktur dem Versuchstiere eigen ist.

Nach Michaelis und Oppenheimer[26] hat das Verdauungsferment, besonders Pepsin die Funktion, die Spezifizität artfremden Eiweisses schnell zu vernichten. Wenn aber das Ferment nicht genug wirkt, wird ein Teil davon unverändert resorbiert. Nämlich wenn grosse Menge Eiweiss enteral eingeführt wird oder die Verdauung nicht genug ist, zeigt sich das Eiweiss im Blut.

Behring[5] gab das Immunserum enteral und stellte fest, dass das Antitoxin im Blut Neugeborener ohne jede Veränderung nachweissbar ist, während sich keine Spur davon bei Erwachsenen sehen lässt.

Römer's[32] Angabe nach ist das Antitoxin im Blute neugeborener Kaninchen nachweisbar, wenn man ihnem Muttermilch des Immuntieres gibt. In Sera Erwachsener aber, wenn man auch ihnen Muttermilch in den Magen einführt, lässt sich keine Spur davon empfinden.

Uhlenhuth,[47] Ascoli,[3] Michaelis, Oppenheimer[26] haben die Feststellung gemacht, das Präzipitin wird nur dadurch erzeugt, dass das Eiweiss parenteral, d.h. subkutan, intravenös, intraperitoneal etc. übergeht. Sonst soll jedes gesunde Serum Präzipitin gegen Muttermilcheiweiss haben, was aber noch nicht experimental bewiesen ist. Wenn aber grosse Menge Eiweiss enteral eingeführt wird, geht artfremdes Eiweiss unverändert durch die Darmschleimhaut ins Blut über und lässt Präzipitin entstehen.

Wie die Ergebnisse der Untersuchungen von Ganghofner[12] und Langer an Katzen beweisen, ist das Eiweiss durch Präzipitation nachzuweisen, wenn es des

Quantums von einviertausendtel Körpergewicht mit Rinder-Antisera von 3000 fachem Präzipitationstiter eingeführt wird. Und obgleich ein Teil enteral eingeführtes Eiweisses bei Neugeborenen ins Blut übergeht, geschieht diese Erscheinung bei Erwachsenen nie. Wenn man artfremdes Eiweiss unter künstlicher Verletzung der Magendarmschleimhaut enteral gibt, kann man es in Sera durch die Präzipitation nachweisen. So muss der Uebergang des enteral eingeführten Eiweisses bei erwachsenen Tieren auf Verletzung der Magendarmschleimhaut oder deren Funktionstörung zurückgeführt werden. Auch wenn grosse Menge artfremdes Eiweiss enteral gegeben wird, geht es auch ins Blut über. Das Uebergehen des artfremden Eiweisses ins Blut veranlasst das Entstehen des Antikörpers. Dabei magern Versuchstiere meistens ab und erkranken, ja es sind solche nicht wenig, die absterben.

Man führt den Anaphylaktogen bei der Anaphylaxie durch subkutane Injektion ein. Aber man kann die Anaphylaxie auch durch Einführung in Vene, Bauchhöhle, Herzbeutel erscheinen lassen. Der Anaphylaktogen kann durch Konjunktiva, Respirationsschleimhaut durchgehen. Und ob man die Anaphylaxie durch enterale Einführung des artfremden Eiweisses entstehen lassen könne, darüber gehen die Meinungen der Autoren auseinander. Nach Doerr und Russ[9] kommt das Durchgehen des Anaphylaktogens durch die Wand des Verdauungskanals ausnahmsweise vor. Man muss dabei aber auch das berücksichtigen, dass ein kleiner Teil des Anaphylaktogens bei der Manipulation zufällig in Luftwege eingeführt werden kann oder die Magenschleimhaut von dem Katheter verletzt werden kann. Aber Micheli[10] konnte die Anaphylaxie durch enterale Einführung grosser Menge Rinder- oder Schafsera entstehen lassen.

Lesne und Dreufus[10] laparotomierten Kaninchen und Hunde und spritzten Anaphylaktogen direkt in den Magen, Dünn- und Dickdarm ein. Unter den Resultaten davon war nur das des Dickdarms positiv. Im allgemeinen geben der Zustand des Verdauungsvorganges von Versuchstieren, die Art des Anaphylaktogens und sein Normalfütterungseiweiss bei der Anaphylaxie grosse Einflüsse auf ihre Empfänglichkeit.

Rosenau und Anderson[10] konnten an Meerschweinchen unter gemischter Fütterung grosser Menge Pferdesera die Fütterungsanaphylaxie entstehen lassen.

Wells und Osborne[52] sensibilisierten Meerschweinchen nach allgemeiner Methode mit Fütterungsphytoeiweiss. Da keine Anaphylaxie dabei geschah, gab Wells darüber an, dass ein Tier bei der Fütterung mit einem Eiweisse anfangs für Anaphylaxie gegen das Eiweiss empfindlich ist, während es unempfindlich wird, wenn die Fütterung lange fortdauert. Wenn man also Anaphylaxie an Tiere in dieser Zustande mit Fütterungseiweiss experimentiert, erfolgt es negativ. Dieser unempfindliche Zustand ist leichter mit pflanzlichem Eiweiss als mit dem tierischen erreichbar.

Nach Uffenheimer und Awerbuch,[46] welche die Beziehung zwischen der Anaphylaxie und der Lebertätigkeit studierten, kann man an Meerschweinchen

gewissermassen den Anfall verkleinern oder anaphylaktischen Tod vermindern, im Vergleich zu den Kontrolltieren, wenn man an jenen Reinjektion in Mesenterialgefässvene oder Vorbehandlung macht. Bei der Reinjektion grosser Menge Eiweiss sieht man fast keinen Unterschied zwischne ihnen und Kontrolltieren. Daher hat die Leber eine bestimmte Funktion auf Eiweiss und kann es im Kreise der Funktion verfügen.

II. Versuchungsmethoden.

Ich will jetzt vom serologischen Standpunkt aus unter Anwendung der Präzipitation und Anaphylaxie untersuchen, ob das enteral eingeführte Eiweiss durch die Wand des Verdauungskanals gesunder Tiere durchgehe und wie die Leber auf das Eiweiss wirkt.

Der Physiologie nach ist die Resorption der Wand des Verdauungskanals von Eiweiss anders als der Vorgang, dass das Eiweiss mit physikalischen Wirkungen, d.h. Druck, Osmose, Diffusion durch das tierische Membran durchgeht. Diese Resorption hängt von der Späzifizität lebender Zellen ab. Und die Leber steht zu dem Stoffwechsel der Kohlenhydrate in enger Beziehung und ist zugleich ein Schutzorgan. Unter Betrachtung der anatomischen Beziehung, dass das von der Wand des Verdauungskanals resorbiertes Eiweiss enthaltende Blut in Mesenterialgefässe eintritt, damit es durch Pfortader, Leber und Lebervene in Hohlvene übergeht, habe ich die Unterschiede untersucht, welche es zwischen den Eiweissgehalten des Pfortader- und Lebervenenbluts im Normalzustand und nach bestimmter Manipulation gibt. Und ich versuchte die Erscheinungen, welche durch direkte Injektion in Pfortader von Eiweiss geschehen, durch Präzipitation und Anaphylaxie, experimental die Wirkungen der Wand des Verdauungskanals und der Leber auf Eiweiss und die Funktion der Leber auf Kohlenhydrate zur Vergleichsuntersuchung. Die Präzipitation ist eine so empfindliche und spezifische Erscheinung, dass das über 1000 fach verdünnte Eiereiweiss, wie Uhlenhuth[6] angab, durch jede chemische Untersuchungsmethode unnachweisbar ist, während durch Präzipitation selbst das 100000 fach verdünnte Eiweiss nachzuweisen ist. Das artfremde Eiweiss kann wohl, auch dasjenige, welches keine chemische Reaktion des Eiweisses geben kann serologisch zu Antigen werden. Es ist im allgemeinen anerkannt, die Anaphylaxie als Nachweisungsreaktion des artfremden Eiweisses sehr empfindlich und spezifisch ist. Nach Rosenau[8] ist die Minimumdosis der subkutanen Injektion als der Sensibilisierung bei der Anaphylaxie mit dem Pferdeserum 0.000001 ccm.

Die Präzipitation und Anaphylaxie sind, wie man aus den obigen Ausführungen leicht verstehen kann, viel empfindlicher und spezifischer als chemisches Mittel der Nachweisung des Eiweisses. Darum will ich sie zu meinem Versuche anwenden. Das Eiweiss, welches ich zum Experiment anwenden will, soll in das pflanzliche und in tierische geteilt werden. Und ich will zuerst über die

Präzipitation des pflanzlichen Eiweisses, das bis jetzt noch nicht genug erforscht bleibt, studieren. Ich will, kurz gefasst, vom serologischen Standpunkt aus die antigenösen Veränderungen des enteral eingeführten, artfremden Eiweisses unter Anwendung der Präzipitation und Anaphylaxie verfolgen.

III. Über die Präzipitation des pflanzlichen Eiweisses.

Kowarski[19] gab an, der die Präzipitation des pflanzlichen Eiweisses 1901 zum ersten Male mit löslicher Weizeneiweisslösung versuchte, dass die Spezifizität des pflanzlichen Eiweisses nicht so deutlich wie die des tierischen ist, da das Serum nicht nur in der Kochsalzextraktlösung des Weizens, sondern auch in den anderen pflanzlichen Eiweisslösung leichte Präzipitation zeigen lässt.

Bertarelli[6] hat über Bohnen und Erbsen untersucht. Nach seiner Angabe reagiert das Bohnenextrakt-Antiserum in seiner 300–3000 fachen Verdünnung positiv, während es in dem Extrakt der anderen Pflanzen von Leguminoson nur bei 60–100 facher Verdünnung positiv reagiert. Da jedes Extrakt-Antiserum von Leguminoson stärker positiv in seiner eigenen Eiweisslösung als in der von anderen Pflanzen reagiert, lässt sich das Eiweiss als Antigen gegen das Antiserum feststellen. Zu diesem Experimente soll man über 500 fach verdunnte Extraktlösung gebrauchen.

Magnus und Fridenthal[24] fanden, dass das Bohnen- und Weizenextrakt-Antiserum spezifisch ist und dass das durch Immunisierung an einem Teile einer Pflanze erhaltene Präzipitin in dem Extrakt anderer Teile von derselben Pflanze, z. B. Frucht, Blatt, Wurzel, positiv reagiert. Darum behaupteten sie, dass die Präzipitation eines Immunserums mehr spezifischer in der Pflanzeneiweisslösung als in der tierischen erscheint.

Gäsis[14] fand, dass das Präzipitin, welches er durch Immunisierung mit dem Extrakt von Weizen, Reis und Leguminoson an Kaninchen zustandekommen liess, sehr hochgradige Spezifizität hat und behauptete, dass sich die Familie sehr nahestehender Pflanzen dadurch unterscheiden lässt.

Magnus[21] stellte fest, dass das durch schwache Immunisierung mit einem Pflanzenextrakt an einem Tiere erhaltene Antiserum nur in dem Extrakt derselben Pflanze die Präzipitation zeigt, aber je höher die Immunität steigt, reagiert das Antiserum auch im Extrakt der nahen Pflanzenfamilie und dann weiter in der aller Pflanzenfamilien, und endlich zeigt sich positive Reaktion in der Lösung aller zu Graminaceoe gehörigen Pflanzen. Darum kann man durch diese Reaktion die Verwandtschaft der Pflanzen kennen.

Wells und Osborne[62] immunisierten Kaninchen mit einem Pflanzeneiweiss und konnten den Antikörper durch Komplementbindung, Präzipitation und Anaphylaxie anerkennen. Das Antiserum hat sehr exakte Spezifizität. Aber je höher die Immunität steigt, je schwächer wird die Spezifizität, und endlich kann es Reaktion dem nahen Eiweisse geben. Bei dieser Immunisierung entstehen zuerst komplementbindende Substanz und dann anderer Antikörper.

Thöni und Thaysen[42] konnten feststellen, dass das in den Getreidearten Roggen, Weizen und Gerste enthaltene Eiweiss lässt sich durch fraktionierte Ausfällung mit Ammonsulfat in zahlreiche verschiedene Esweisskörper zerlegen. Die mit einzelnen dieser Eiweisskörper hergestellten präzipitierenden Antisera zeigen eine bedeutende stärker ausgeprägte Spezifizität als die mit dem Gesammteiweiss erzeugten Sera.

Ich will nun von dem Präzipitinogen etwas sagen, welchen oben beschriebene Autoren bereiteten. Kowarski[19] setzte 50 g Weizenmehlen 150 ccm physiologische Kochsalzlösung zu, schüttelte sie im Kolben und filtrierte nach halbe Stunde langem Stehenlassen, bis die Filtrat klar wurde. Sie enthielt 0.5% Eiweiss, gemessen nach Kjerdahlscher Methode.

Gäsis[14] machte Getreidemehle in 10% Kochsalzlösung 24 Stunden lang ein, liess sie im Schütterungsapparat 4 Stunden lang liegen und filtrierte, bis sie klar wurde. Nach Dialysierung und Entsalzung nahm er ausgefallenes Globulin durch Filtration ab und liess lösliches Eiweiss, welches das Filtrat enthielt, durch Alkohol oder verdünnte Essigsäure koagulieren. Er trocknete dann diese Fraktion und pulversierte sie, durch Zusatz physiologischer Kochsalzlösung zu 200 facher Lösung zu machen.

Thöni und Thaysen[42] versuchten genaueste Untersuchungen neuerer Zeit. Sie fügten Roggen-, Weizen-, Gersten- und Hafermehlen physiolagische Kochsalzlösung im Vergleich 1 : 4 zu und liessen sie 24 Stunden lang nach Zusatz des Toluols stehen, um sie dann durch Filtration klar zu machen. Durch intravenöse Einspritzung dieses Extraktes konnten sie nur schwaches Antiserum bekommen. Da fügten sie dieser Extraktlösung chemisch reines Ammonsulfat bis zur Sättigung, d.h. 78% zu, um ganzes Eiweiss zu fraktieren. Dann sammelten sie die Fraktion durch Zentrifugierung und trockneten, um das Ammonsulfat zu entnehmen. Diese 50–100 fache, physiologische Kochsalzlösung lässt, wenn man Kaninchen mit ihr immunisiert, bedeutend Präzipitin entstehen. Als sie das Pflanzenextrakt mit Fraktion von Ammonsulfat halb oder voll sättigten, bekamen sie Globulin und Albumin. Mit 1% Lösung dieser Eiweissstoffe immunisierten sie Kaninchen und bekamen hochgradiges Präzipitin. Und als sie weiter diese Extraktlösung mit Ammonsulfat teilweise wiederholt fraktierten, kamen verschiedene Eiweissarten zustande, und das Antiserum einzelner Eiweissarten zeigte eine bedeutend stärker ausgeprägte Spezifizität als die mit dem Gesammteiweiss erzeugten Sera.

Ich versuche die Präzipitation des Bohneneiweisses, da sie in enger Beziehung mit meinen Experimenten steht. Und nachdem es überzeugt ist, dass dieses Bohneneiweiss für mein Studium zweckmässig ist, will ich weiter meine experimentalen Untersuchungen anfangen.

1. Über die Kaninchenimmunisierung mit Bohnenextrakt.

A. Über die Herstellung des Antigens.

Ich wasche 100 g allen Zusatz entnommene Bohnen mit sterilisierter physiologischer Kochsalzlösung mehreremal, zerreibe sie in einer grossen Reibschale nach eine Nacht langer Einmachung in 400 ccm physiologischer Kochsalzlösung, mache in einem sterilisierten Kolben streng neutral und lasse nach Zusatz des Toluols 24 Stunden lang stehen. Dann mache ich sie durch Filtration klar. Dieses Extrakt reagiert neutral und ihre Eiweissreaktion in Biuret, Millon, Xanthoprotein etc. ist positiv. Wenn dieses Extrakt gekocht wird oder dem Salpeter- oder Essigsäure zugesetzt wird, so entsteht Niederschlag, welcher 0.6168 Eiweiss enthält, gemessen nach Kjedahlscher Methode.

B. Gibt es Normalpräzipitin gesunder Kaninchen und Meerschweinchen gegen Bohnenextrakt?

Zuerst will ich einiges von Normalpräzipitin sagen. Kraus[22] fügte zuerst Ricin dem tierischen Serum zu und sah Präzipitation geschehen. Landsteiner, Raubitschek, Eisler und Portheim[22] auch versuchten Normalpräzipitin gegen Pflanzenextraktlösung. Ascoli[2] sagte, das Tierserum habe sowohl andere Antikörper als auch eigene verschiedene Präzipitine. Nach Wilenko[51] zeigt das Tierserum für Ricin, Abrin, Crocin und Bohnenextrakt Präzipitation, welche mit Vogelserum am hochgradigsten ist. Und die Pflanzenextrakte zeigen Präzipitation nicht nur durch Zusatz der Hemoglobin-, Serumalbumin-, Eieralbuminlösung, sondern auch ineinander

Wenn ich nach oben erwähnter Methode erhaltenes Extrakt mit sterilisierter physiologischer Kochsalzlösung 150 fach verdünnte und damit Präzipitation der Sera der 10 Kaninchen wie 10 Meerschweinchen untersuchte, war sie immer negativ. Aber wenn diese Extraktlösung Säurereaktion gibt, ist diese immer postitiv. Die Bohnenextraktlösung gibt eigentlich ziemlich starke Säurereaktion. Wenn man aber dieses Antigen, welches Säurereaktion gibt, mit Alkali streng neutralisiert, ist die Präzipitation negativ. Daraus lässt sich klar machen, dass die Erscheinung nicht wirkliche Präzipitation, sondern die Erscheinung der Säurereaktion auf Eiweissstoff ist. Und ich bemerkte, wie Wilenko,[51] das hämolysierte Serum, welches Präzipitation zeigt. Daraus wird es klar, dass man so vorbereiten soll, wie die Pflanzenextrktlösung zur Untersuchung immer streng neutral reagiert und das präzipitierende Serum absolut klar ist.

C. Über die Methode der Kaninchenimmunisierung und Präzipitation des Immunserums.

Die Immuntiere und die Immunisierungsmethode waren wie bei allgemeiner Immunisierung. Ich injizierte Kaninchen, wie im allgemeinen, die auf oben genannte Weise erhaltene 150 fache Bohnenextraktlösung intravenös als Antigen.

Bei achtmaligen Injektionen, deren Dosis von 0.5 bis 10 ccm allmählich vermehrt wurde, reagierte es in ungefähr 3000 facher Antigensverdünnung meistens positiv. Im Verlaufe dieser Immunisierungen verminderte sich das Körpergewicht der Kaninchen. Dann injizierte ich ein und dasselbe Kaninchen fortdauernd grosse Menge Antigen, doch konnte ich keinne Steigerung des Präzipitationtiters sehen. Ich immunisierte 5 Kaninchen. Am 7 ten Tage nach der Injektion entnahm ich ihnen etwas Blut aus Carotis und bekam ein absolut durchsichtiges Serum, welches ich ohne Zusatz der Karbolsäure in einer sterilisierten Ampulla in dem Eiskasten aufbewahrte, um damit Präzipitation zu untersuchen. Die Versuchsmethode war Fornetsche Ringprobe[12] mittelst kleineres Reagenzglases, d. h. man überschichtet Antigen auf Serum, so entstehen sofort weisse Scheiben positiv, wie sie bei Hellerscher Reaktion[40] entstehen, an den Berührungsflächen. Diese weissen Scheiben erhöhen ihren Grad nach einigen Minuten, erweitern sich allmählich und fallen endlich auf Grund des Gläschens aus. Diejenige, welche nach einviertel Stunde erst zustandekommen, sind negativ, und dieselbe, welche nach 5 Minuten erst erscheinen, sind unsicher. Und die unspezifischen, weissen Scheiben haben keine deutliche Grenze an ihrer unteren Fläche, und zwar auch der Grad erhöht sich nicht auch nachher.

D. Über die Präzipitation des Immunserums gegen andere Extrakt- und Tiereiweisslösungen.

Ich versuchte Präzipitation des obigen Antiserums in anderen Pflanzenextrakt- und Tiereiweisslösungen. Es reagierte auf Antigen der zu Leguminoson gehörigen Pflanzen, z. B. Schwarzbohnen, Erbsen, Kleinrotbohnen (Phaseous rediatus) positiv, und zwar in Extraktlösung der Schwarzbohnen zeigt es sich beinahe gleichgradig positiv, aber es erscheint in der Bohnen und Kleinrotbohnen nur leichgradig positiv. Im Gegensatz dazu war sie ganz positiv, während es in Pflanzeneiweisslösungen der Familie Graminaceoe, z. B. Gersten, Weizen, Reis immer negativ reagiert, was mit der Angabe von Magnus[24] ganz übereinstimmt. Unter tierischen Eiweissarten ist sie gegen Antigen des Normalpferdserums, Rinderserums, der Tiereiweisslösung ganz negativ. Daher ist es gewiss, dass dieses Immunserum eine Spezifizität gegen Antigen anderer Pflanzenfamilie hat. Ich gaube also, es sei durchaus tadellos, mein antigen als Untersuchungsmittel experimentellen Studiums zu benutzen, wenn auch der Präzipitationstiter dieses Immunserums etwas schwach ist.

2. Über die Immunisierung mit Eiweiss, welches durch Ammonsulfat vollgesättigt ausfällt.

Da die auf oben erwähnte Weise bereitete Bohnenextraktlösung noch etwas ausser Eiweiss enthielt, fügte ich ihr langsam chemisch reines Ammonsulfat bei, um es damit zu sättigen, und liess 24 Stunden lang stehen. Dann filtrierte ich das Eiweiss, welches koagulierte und ausfällte. Nachdem Entwässern machte ich 100 fache Lösung davon, welche ich in dem Dialysierungsack 7 Tage in fliessendem

Wasser dialysierte, um zu entsalzen. Dabei gab es auch solches Eiweiss, welches frakzierte. Nun fügte ich dieser Lösung Kochsalz zu, bis sie zu physiologischer Kochsalzlösung wurde, und liess nach Zusatz des Toluols eine Nacht lang im Eiskasten stehen. Und zuletzt bekam ich durch Filtrierung klare Lösung, diese reagierte neutral, und ihre Biuretreaktion und chemische Reaktion auf Eiweiss sind positiv. Weder Schwefelsäure noch Ammonium waren nicht nachzuweisen. Ich immunisierte Kaninchen nach oben genannter Methode mit dieser Lösung. Die Sera der 5 immunisierten Kaninchen hatten alle ungefähr 3000 fachen Präzipitationstiter. Und ich spritzte denselben Kaninchen mit grosser Menge desselben Antiserums ein, doch sah ich keine Steigerung des Titers. So kann man mit dieser Eiweisslösung hochgradigeres Immunserum als mit Extraktlösung durch Kaninchenimmunisierung bekommen. Und dass Antigen in Normalsera der Kaninchen und Meerschweinchen negative Präzipitation gibt und wie sich die Präzipitation des Immunserums mit anderen Pflanzenextrakt- und Tiereiweisslösungen verhält, stimmt mit den Ergebnissen der oben gezeichneten Experimente genau überein.

3. Über die Kaninchenimmunisierung mit dem teilweise frakzierten Bohneneiweiss.

Über die Klassifikation des Pflanzeneiweisses gehen bischerige Untersuchungsberichte auseinander. Osborne Campbell[27], einer der grossen Autoren in diesem Kreise, klassifizierte das Pflanzeneiweiss in folgende drei Bestandteile: 1) wasserlösliches Leukosin, 2) Edestin, in Kochsalzlösung löslich und 3) 75% Hardein, in Alkohol löslich. Aber es gibt andere Autoren welche behaupten, dass gewisse Pflanzen noch anderes enthalten. Bei meiner Klassifikation des Eiweisses wandte ich die Fraktion- und Dialysierungsmethode, wie die Klassifikatiomethode des Serumeiweisses, welche am einfachsten und zweckmässigsten ist.

Erste Fraktion (Globulin) — Der physiologischen Kochsalzlösung des Pflanzeneiweissextraktes gibt man eine bestimmte Menge der chemisch reinen Neutralsättigungs-Ammonsulfatlösung sehr langsam tropfenweise und lässt sie 24 Stunden lang stehen. Wenn man den so entstandenen, koagulierten Niederschlag filtriert, bekommt man das Eiweiss, welches dem Serumeiweiss Globulin entspricht.

Zweite Fraktion (Albumin) — Dem obigen Filtrat fügt man grosse Menge kristallisiertes Ammonsulfates zu, um zu sättigen und frakzieren. Wenn man den nach 24 stündigem Stehenlassen entstandenen, koagulierten Niederschlag filtriert, erhält man das Eiweiss, welches dem Serumeiweiss Albumin entspricht. Den so zustandegekommenen Niederschlag drückt man zwischen zweien Filtrationpapieren, um genug zu entwässern, und löst ihn im Verhältnis 1% in sterilisierter physiologischer Kochsalzlösung. Dann dialysiert man sie in einem Dialysierungssack 7 Tage lang in fliessendem Wasser, um genug zu entsalzen, und macht durch Zusatz des Kochsalzes physiologische Kochsalzlösung. Und da nicht nur Schwefelsäure sondern auch Ammonium nicht nachzuweisen ist, kann man anerkennen, dass diese Eiweisslösung nie Ammonsulfat enthält. Diese beiden Eiweisslösungen

reagieren neutral, und ihre Farbenbiuretreaktion ist positiv. Und wenn ich Präzipitation des Normalserums als Antigen mit Bohneneiweisslösung vor Beginn der Kaninchenimmunisierung untersuchte, war sie immer negativ. Auch in Sera wie in Antigen gegeneinander erfolgte es negativ. Ich immunisierte mit dieser Lösung nach derselben Methobe wie oben. Und wenn ich Präzipitation des dadurch erhaltenen, klaren Serums an 3 Kaninchen untersuchte, war der Präzipitationstiter der Albumin-Antisera ungefähr 8000 fach, während derselbe der Globlin-Antisera etwas niedrig, d. h. ungefähr 5000 fach war. Die Beziehungen der Präzipitation dieser Antisera mit anderen Pflanzen- und Tiereiweisslösungen waren ganz gleich den oben beschriebenen Ergebnissen. Also wenn man Kaninchen mit dieser Eiweisslösung immunisiert, erhält man hochgradigeres Immunserum als in vorhergehenden zeigte.

4. Über die Immunisierung mit Koktopräzipitinogen.

Dass ich diesen Versuch unter Anwendung des Kokto-präzipitinogens, welcher von Ascoli zuerst studiert und von Prof. Dr. Torikata empfohlen wurde, beobachte, ist für meine weiteren Untersuchungen notwendig. So kochte ich Bohnen halbe Stundelang, bereitete nach derselben Methode das Antigen und dann immunisierte Kaninchen auf dieselbe Weise. Als ich Präzipitation daran untersuchte, sah ich auch dieselbe Resultate. Diese Methode hat einen Vorteil, Eiweiss leichter zu erhalten, als die, welche ich oben genannt habe. Da das präzipitierende Antiserum gegen pflanzliches Eiweiss, besonders Bohneneiweiss verhältnismässig hochgradig ist und eine Spezifizität hat, habe ich dieses als Untersuchungsmittel meines Experimentes benutzt.

IV. Geht das enteral eingeführte Eiweiss durch die Wand des Verdauungskanals durch?

Die Hauptzüge der Untersuchungsergebnisse von Autoren über das Schicksal des enteral eingeführten Eiweisses habe ich bereits in der Einleitung geschildert. Jetzt will ich prüfen, ob das enteral eingeführte Eiweiss durch die Wand des Verdauungskanals durchgehe und im Blut nachweisbar sei, und wie die Leber auf dieses Eiweiss wirke.

1. Über Untersuchungen des Antigens durch Präzipitation.

Ich wollte zuerst die zeitlichen Beziehungen des Übergangs vom artfremden Eiweiss ins Blut betrachten, wenn es enteral eingeführt wird. Zu diesem Zwecke gab ich den Meerschweinchen im hungrigen Zustande das Eiereiweiss im Verhältnisse 2 ccm zu 100 g Körpergewicht. Und als ich Präzipitation mit dem durch Herzpunktion gewonnenen Blut und dem Antiserum untersuchte, konnte ich feststellen, dass die Reaktion nach einviertel Stunde positiv ist und allmählich bedeutender wird, bis sie nach 2–3 Stunden die höchste Grenze erreicht und dann wieder

schwächer wird. Nun will ich unter Erwägung des von der Wand des Verdauungskanals abgesonderten Fermentes über Pflanzenfresser und Raubtier getrennt prüfen.

A. Bei dem Pflanzenfresser.

An Kaninchen und Meerschweinchen untersuchte ich mit dem tierischen Eiweiss und dem pflanzlichen getrennt.

a. Experiment mit tierischem Eiweiss.

Ich gebrauchte präzipitierendes Antiserum vom 10000 fachen Präzipitationstiter, welches ich durch Immunisierung mit Eiereiweiss erhielt. Das Eiweiss, welches ich enteral einführte, war Eiereiweiss. Das verdünnte ich in physiologische Kochsalzlösung und gab bestimmte Menge davon dem Versuchstiere in die Mundhöhle mit der Pipette. Nach Verlauf einer bestimmten Zeit entnahm ich das Blut aus der Pfortader und Lebervene durch Herzpunktion und regelmässige Laparotomie. Da die Entnahme des Blutes aus der Lebervene im allgemeinen, besonders bei Meerschweinchen aus anatomischem Zustande ziemlich schwer war, gelang es mir oft nicht. Ich untersuchte Präzipitation, welche zwischen dem befreiten Serum und dem Eiereiweiss-Antiserum erscheint. Aus den Resultaten dieser Untersuchung, welche Tabelle I zeigt, kann man folgendes schliessen. Man kann durch Präzipitation nachweisen, dass das tierische Eiweiss durch die Wand des Verdauungstraktus durchgeht, von ihr resorbiert wird und ins Blut übergeht, wenn eine bestimmte Menge davon enteral eingeführt wird. Dass der Eiweissgehalt nach Durchgang der Leber demselben vor Durchgang verschieden ist, lässt sich dadurch verstehen, dass die Präzipitation nur gegen das Pfortaderblut-Serum positiv erscheint, während sie gegen Lebervenenblut- und Herzblut-Serum negativ erscheint, oder dass es Unterschied zwischen den Graden der Reaktion gibt, wenn sie gegen alle Sera positiv erscheint. Die Ursache des Unterschiedes von Reaktionen kann man vielleicht der Funktion der Leber zuschreiben, obgleich ich nicht Ecksche Fistel herstellte und bewies. Denn kein Organ befindet sich als die Leber zwischen der Pfortader und Lebervene. Im allgemeinen ist es klar, dass die Resorption nach eingeführter Menge, Zustand der Magen- und Darmtätigkeit, deren Inhaltmengen und Ablauf nach der Einführung verschieden ist, wenn das Eiweiss enteral eingeführt wird. Daher ist es sehr schwer, die absolute, zum Erscheinen der Präzipitation nötige Menge des Eiweisses zu bestimmen.

b. Experiment mit pflanzlichem Eiweiss.

Als präzipitierendes Immunserum gebrauchte ich das Bohneneiweiss-Antiserum. Zur enteralen Einführung benutzte ich das 100 fache Bohnenextrakt. Die Resultate der nach der wie oben erwähnten Methode ausgeführten Experimente zeigen, wie Tabelle II lehrt, das man dieselbe Ergebnisse mit pflanzlichem Eiweiss wie mit dem tierischen hat, nur dass der Präzipitationtiter des Antiserums bei dem pflanzlichen niedriger ist und der Grad der Verdünnung von enteral einge-

führter Eiweisslösung gross ist. Da die Menge des Eiweisses folglich wenig ist, ist die Erscheinung der Präzipitation sehr leicht. Bei diesen meinen Experimenten sind alle Kontrollversuche negativ. Darum sind die Ergebnisse der Präzipitation in diesem Artikel die Erscheinung, die Spezifizität zu behalten.

B. Bei dem Raubtier.

Auch diese Untersuchung führte ich aus, indem ich das tierische Eiweiss und das pflanzliche als Eiweiss zur Einführung benutzte. Zum Versuche gab ich den Hunden bestimmte Menge artfremden Eiweisses, z. B. Eiereiweisses und Bohnenextraktes enteral und entnahm regelmässig Herz-, Lebervene- und Pfortaderblut nach Verlauf bestimmter Zeit. Durch Untersuchungen über Präzipitation gegen ihr Serum und Antiserum, deren Resultate Tabelle III zeigt, konnte ich feststellen, dass die Ergebnisse der Untersuchungen über den Hund mit denselben über den Pflanzenfresser übereinstimmt. Bei diesen meinen Experimenten waren auch alle Kontrollversuche negativ. Wenn artfremdes Eiweiss, kurz gefasst, mehr als eine bestimmte Menge enteral eingeführt wird, geht ein Teil davon, die präzipitinogenöse Eigenschaft behaltend, durch die Wand des Verdauungskanals im Normalzustande durch und ins Blut über. Auf dieses resorbierte Eiweiss hat die Leber gewisse Tätigkeit und, wenn es über die Grenze ihrer Tätigkeit eingeführt wird, geht ein Teil davon in den Körperkreislauf über, was durch Präzipitation sowohl bei dem Raubtier als auch bei dem Pflanzenfresser nachweisbar ist.

2. Über Zustandekommen des Präzipitins.

Dass ein Teil artfremden Eiweisses, wenn es mehr als eine bestimmte Menge enteral eingeführt wird, von der Wand des Verdauungstraktus resorbiert wird und ins Blut übergeht, kann man durch Präzipitation nachweisen, wie ich im vorhergehenden gesagt habe. Nach der Immunologie soll der Antikörper gegen artfremdes Eiweiss in Vivo erzeugt werden, wenn dieses wiederholt ins Blut übergeht. Ich will jetzt prüfen, ob das ins Blut übergegangene Eiweiss zu Präzipitinogen werden und Präzipitin erzeugen kann. Ascoli und Uhlenhuth[27] haben bereits experimentiert, dass das Präzipitin entsteht, wenn man Kaninchen mit Eiereiweiss überfüttert. Und Cantacuzene[27] hat positive Reaktion gegen das Pferdeserum erfahren. Ich wollte untersuchen, ob man durch Präzipitation den Antikörper nachweisen könne, welcher dadurch erzeugt wird, dass man Kaninchen Eiereiweiss oder Pferdeserum wiederholt und fortdauernd enteral gibt. Bevor ich zu immunisieren anfing, hatte ich vorher festgestellt, dass die Präzipitation des Normalkaninchenserums gegen Antiserum negativ ist. Und bei Experimenten wiederholte ich immer mit grösster Vorsicht, artfremdes Eiweiss mittelst des Gummikatheters in den Magen einzuführen. Die Resultate davon, sind wie folgendes; drei Kaninchen wurden mit Normalpferdeserum in aufsteigender Dosis von 40 cc. bis auf 80 cc. bzw. in sechsmal per os eingeführt. Der Präzipitintiter dabei zeigt bei einem Kaninchen 700, bei anderem 1000 und bei letztem wieder 1000. Zwei Kaninchen, die mit Eiereiweiss gefüttert

worden sind und zwar in Menge von 10 cc. an bis 25 cc. in sechsmal. Dabei zeigt der Präzipitintiter 50 und 70. Daraus kann man schliessen, dass man durch Präzipitation den Antikörper nachweisen kann, welcher dadurch im Körper erzeugt wird, dass man eine bestimmte Menge artfremden Eiweisses wiederholt enteral einführt. Wenn der Antikörper nicht so bedeutend zustandekommt, ist das Präzipitin, welches durch Pferdeserum als Antigen erzeugt wird, viel hochgradiger als das, welches durch Eiereiweiss als Antigen erzeugt wird.

3. Über Untersuchungen des Antigens durch Anaphylaxie.

Ich will jetzt durch Anaphylaxie untersuchen, ob ein Teil artfremden Eiweisses unverändert durch die Wand des Verdauungstraktus durchgeht, von ihr resorbiert wird und ins Blut übergeht, wenn es mehr als bestimmte Menge enteral eingeführt wird.

A. Über Anaphylaxie durch enterale Sensibilisierung.

Ich gab Meerschweinchen eine bestimmte Menge Normalpferdeserum oder Eiereiweiss enteral und spritzte wieder nach Verlauf einer Inkubationszeit eine bestimmte Menge Pferdeserum oder Eiereiweisslösung in Fussvene ein. Dann untersuchte ich ihre Anaphylaxie. In dieser Untersuchung sind fünf Meerschweinchen mit 5 cc. Pferdeserum und andere fünf mit 5 cc. Eiereiweiss per os sensibilisiert. Diesen sind nach zwei Woche mit entsprechender Antigene und zwar in Menge je 5 cc. in Fussvene reinjiziert. Vier von ersten Gruppe, die mit Normalpferdesum behandelt waren, gingen typisch anaphylaktisch und einer in mässiger Grad. Bei Eiereiweissgruppe zwei typisch positiv, einer mässig und andere zwei in leichter Symptome. Dabei konnte ich auch anerkennen, dass die Präzipitation gegen das Herzblut des Versuchstieres immer positiv war, dem ich vorher artfremdes Eiweiss enteral eingeführt hatte. Aus den Ergebnissen dieser Untersuchungen kann man schliessen: die Anaphylaxie entsteht auch dadurch, dass man Meerschweinchen durch enterale Einführung artfremden Eiweisses vorbehandelt, wie bei der Sensibilisierung durch subkutane Injektion, obgleich die Menge des Eiweisses bei der enteralen Sensibilisierung ziemlich gross sein soll.

B. Über Anaphylaxie der mit dem das enteral eingeführtes Eiweiss enthaltenden Serum sensibilisierten Meerschweinchen.

Die Menge des Eiweisses, welches zur subkutanen Injektion als Sensibilisierung nötig ist, ist geringfügig. Ihre Minimumdosis ist 0.000001 (Rosenau und Andersen) — 0.00001 ccm Pferdeserum (Besredka und Steinhardt), 0.00001 ccm Rinderserum (Pfeiffer) oder 0,0000005 mg Kristallalbumin (Wells). Ich gab Kaninchen im Hungerzustande grosse Menge Pferdeserum oder Eiereiweiss enteral und entnahm nach 1 Stunde durch regelmässige Laparotomie Pfortader- und Lebervenenblut. Die Präzipitation beider Sera gegen Antiserum war immer positiv. Als ich an vier und zwanzig Meerschweinchen eine bestimmte Menge dieses Serums subkutan

injizierte und nach Verlauf einer Inkubationszeit in Fussvene reinjizierte, um zu untersuchen, ob die Anaphylaxie entstehe, sah ich an fünf Meerschweinchen leichten anaphylaktischen Anfall, welche mit Pfortaderblutserum sensibilisiert wurden. Daher kann man auch durch Anaphylaxie nachweisen, dass, wenn Pferdeserum oder Eiereiweiss als artfremdes Eiweiss Kaninchen und Meerschweinchen mehr als eine bestimmte Menge enteral eingeführt wird, ein Teil davon, die anaphylaktogenöse Eigenschaft behaltend, durch die Wand des Verdauungskanals durchgeht, von ihr resorbiert wird und ins Blut übergeht. Aus den Resultaten der vorhergehenden Experimente kann man wie folgt schliessen: wenn artfremdes Eiweiss den Versuchstieren mehr als eine bestimmte Menge enteral eingeführt wird, geht ein Teil davon, die antigenöse Eigenschaft behaltend, durch die Wand des Verdauungstraktus im Normalzustand durch, wird von ihr resorbiert und geht ins Blut über. Und die Leber hat eine bestimmte Tätigkeit auf dieses Eiweiss. Wenn das Eiweiss über die Grenze ihrer Tätigkeit von der Wand des Verdauungstraktus resorbiert wird, geht ein Teil davon durch die Leber in den Körperkreislauf über, und bei der Wiederholung dieser Manipulation kommt der Antikörper gegen dieses Antigen in Vivo zustande.

V. Geht das Eiweiss durch die Wand des Verdauungstraktus der Tiere unter Normalfütterung durch?

Es ist aus den oben erwähnten Experimenten klar, dass ein Teil artfremden Eiweisses, wenn es mehr als eine bestimmte Menge enteral künstlich eingeführt wird, behaltend die antigenöse Eigenschaft, durch die Wand des Verdauungstraktus durchgeht, von ihr resorbiert wird und ins Blut übergeht. Wie steht also mit dem Eiweiss, welches das Tier unter Normalfütterung aufnimmt? Ich will jetzt den Versuchstieren unter Normalfütterung Pfortader-, Lebervenen- und Herzblut durch regelmässige Laparotomie entnehmen und über ihren Präzipitinogen gegen das Immunserum unter Anwendung der Präzipitation untersuchen. Auch hier will ich über Pflanzenfresser und Raubtier getrennt prüfen.

A. Über den Pflanzenfresser.

Zuerst will ich an Kaninchen und Meerschweinchen experimentieren, welche mit Normalfutter, z. B. Tohfu-no-Kara, Weizenkleien, etwas Gemüse, Mohrrübe gefüttert wurden, ob ein Teil des im Futter enthaltenden Bohneneiweisses unverändert durch die Wand des Verdauungstraktus durchgeht und von ihr resorbiert wird. Aber ich hatte vorher bei der Vorprüfung festgestellt, dass die Präzipitation des Bohneneiweiss-Antiserums gegen gekochtes Bohnenextrakt oder Tohfu-no-Kara-Extrakt positiv ist. Als ich mit Bohneneiweiss-Antiserum vom 3000 fachen Präzipitationstiter über Präzipitation gegen die Sera des Herz-, Lebervenen- und Pfortaderbluts untersuchte, welche den Versuchstieren im Hungerzustand und nach der Fütterung mehreremal entnommen wurden, war die Präzipitation des Pforta-

aderbluts, welches bald nach der Fütterung entnommen wurde, meistens positiv, wie Tabelle IV zeigt, während die desselben, welches im Hungerzustand entnommen wurde, negativ war. Und die Präzipitation gegen Sera, welche aus Herz- und Lebervenenblut befreit wurden, war immer negativ. Die Resultate aller Kohtrollversuche waren negativ. So wurde es klar, dass die Präzipitation eine spezifische Reaktion ist. Aus Vorhergehenden kann man wie folgends schliessen: Bei Kaninchen und Meerschweinchen geht ein Teil des von ihnen aufgenommenen Bohneneiweisses, die präzipitinogenöse Eigenschaft behaltend, durch die Wand des Verdauungstraktus durch. Und das resorbierte Eiweiss wird durch Tätigkeit der Leber in dieser fixiert und assimiliert. Hier kann man aber in Verdacht nehmen, ob es auf eine zu grosse Verdünnung resorbierten Eiweisses zurückgeführt werden kann, dass die Reaktion des Herzblutes negativ war. Denn, wenn aufgenommenes, artfremdes Eiweiss immer ins Blut eingeführt wird, soll nach der Immunologie der Antikörper gegen dieses Eiweiss im Körper entstehen, was aber im Verdauungs-, Resorptions- und Assimilationsvorgange unrationell ist. Die Untersuchungen über Präzipitation des Antikörpers gegen Bohneneiweisslösung an 20 Kaninchen und 20 Meerschweinchen unter Normalfütterung waren immer negativ.

B. Bei dem Raubtier.

Nachdem ich Hunde als Versuchstiere mit einem gemischten Futter des Reises und Pferdfleisches gefüttert hatte, entnahm ich nach verschiedenen Zeiträumen das Herz-, Lebervenen- und Pfortaderblut nach der regelmässigen Methode und untersuchte ihre Präzipitation gegen das Antiserum vom 5000 fachen Präzipitationstiter. Die Resultate dieses Experimentes zeigt Tabelle V. Es ist ja im allgemeinen bekannt, das Antiserum, welches durch die Immunisierung mit Serum eines anderen Tieres erzeugt wurde, lässt dieselbe spezifische Präzipitation gegen Serum und Muskelextrakt desselben Tieres erscheinen.[23] Bei diesen Experimenten waren alle Kontrollversuche negativ. Aus den Resultaten der oben erwähnten Untersuchungen wurde es klar, dass die Präzipitation des Pfortaderblutserums, welches binnen 1 Stunde nach der Normalfütterung entnommen wurde, auch gegen Antiserum ist positiv, während die des Lebervenen- und Herzblutes immer negativ ist. Im Hungerzustande aber ist auch die des Pfortaderblutes negativ. Daher kann man das feststellen, dass ein Teil artfremden Eiweisses bei dem Raubtier wie dem Pflanzenfresser unter Normalfütterung durch Lebertätigkeit in der Leber fixiert und assimiliert wird, wenn er auch, die präzipitinogenöse Eigenschaft durch die Wand des Verdauungstraktus behaltend, durchgeht und ins Blut übergeht. Auch bei diesen Untersuchungen bemerkte ich nicht wie bei dem Pflanzenfresser, das Normalpräzipitin entstehen. Aus den oben beschriebenen Versuchen wird folgende Feststellung gemacht: Bei den Tieren unter Normalfütterung geht ein Teil des aufgenommenen, artfremden Eiweisses unverändert durch die Wand des Verdauungstraktus durch, wird von ihr resorbiert und geht durch Mesenterialvenen in die Pfortader über, aber der ganze teil dieses Eiweisses wird von der Leber resorbiert

und assimiliert und geht nicht in den Körperkreislauf über. Nämlich der Grad des Durchganges vom artfremden Eiweiss unter Normalfütterung geht gewöhnlich nicht über die Grenze der Lebertätigkeit.

VI. Über die Veränderungen der Antigenität artfremden Eiweisses in der Leber.

In dem letzten Artikel habe ich darüber experimentiert, dass ein Teil des enteral eingeführten, artfremden Eiweisses unverändert durch die Wand des Verdauungstraktus im Normalzustande durchgeht und die Leber eine bestimmte Funktion auf das resorbierte, artfremde Eiweiss ausübt. Hier will ich die antigeösen Veränderungen des artfremden Eiweisses in der Leber unter Anwendung der Präzipitation und Anaphylaxie näher untersuchen.

1. Über Präzipitation gegen Serum des mit artfremdem Eiweiss in die Pfortader injizierten Meerschweinchens.

Indem ich Meerschweinchen als Versuchstier benutze, will ich mit Eiereiweiss als dem tierischen Eiweiss und Bohneneiweiss als dem pflanzlichen getrennt versuchen.

A. Experiment mit tierischem Eiweiss.

Als präzipitierendes Immunserum gebrauche ich Eiereiweiss-Antiserum vom 10000 fachen Präzipitationstiter. Zuerst injiziere ich eine bestimmte Menge des mit physiologischer Kochsalzlösung verdünnten Eiereiweisses an dem einen Meerschweinchen in die Fussvene und am anderen in die Pfortader. Und nach Verlauf einer bestimmten Zeit entnehme ich etwa Blut durch Herzpunktion, um die Mengen des in beiden Sera enthaltenen, artfremden Eiweisses zu vergleichen. Die Injektion in Fussvene wird wie folgt ausgeführt: man streckt zuerst ein Hinterbein des Meerschweinchens aus und schneidet die Haut an der Aussenseite hinter dem Kniegelenk ab, damit man dann die Nadel in die entblösste Fussvene hineinsteckt und die Lösung langsam nach Blutstrom einspritzt. Nach der Injektion muss die Stelle gedrückt, um nicht zu verbluten, verbunden werden. Und bei Injektion in Pfortader wird es so behandelt, dass man regelmässig den Bauch aufmacht und den Darmkanal aus der Bauchhöhle herauszieht, bis sich die Pfortader sehen lässt. Und nachdem man die Eiweisslösung nach Blutstrom der Pfortader eingespritzt hat, reponiert man den Darmkanal sogleich, schliesst die Bauchwand mit dem regelmässigen Naht zu und überstreicht mit Tinkturajodati. Tabelle VI zeigt die Resultate der nach oben erwähnter Methode gemachten Untersuchungen. Als ich als Kontrollversuch die Präzipitation des Serums gesunder Meerschweinchen gegen Antiserum untersuchte, war sie immer negativ. Aus den Resultaten der oben gezeichneten Experimente versteht man, dass das in Fussvene des Meerschweinchens eingespritzte, tierische Eiweiss in dem Masse vorkommt, dass sich die

Präzipitation in seinem Serum zeigt, während sie, wenn dieselbe Menge Eiweiss in Pfortader eingespritzt wird, negativ ist. Aber die Injektionsmenge des Eiweisses eine gewisse Grenze. Und wenn das Eiweiss über diese Grenze eingeführt wird, lassen beide Sera endlich Präzipitation erscheinen. Als ich über Präzipitation gegen beide Sera untersuchte, indem ich sie allmählich verdünnte, erkannte ich an, wie Tabelle VII zeigt, dass es grosse Unterschiede zwischen den Stärken beider Präzipitationen gibt und die Präzipitation gegen das durch Injektion in Fussvene erhaltene Serum stark erscheint. Unterschied der Titer beider Präzipitationen sind im Verhältnisse ca. 1 : 4. Dieser Unterschied stimmt fast mit dem der Mininummengen des Eiweisses überein, welches zur Erscheinung beider Präzipitationen nötig ist. Aber bei diesen Versuchen soll noch die Zeit in Berücksichtigung genommen werden. Nämlich gibt es ja eine Frage, ob das in Pfortader eingespritzte Eiweiss etwas später im Blut erscheinen, da es durch die Leber durchgeht. So injizierte ich eine bestimmte Menge artfremden Eiweisses an den beiden Versuchstieren in die Pfortader und entnahm Blut nach verschiedenen Zeiträumen, um Präzipitation zu untersuchen. Aus diesen Experimenten konnte ich feststellen: wenn das Serum, welches 10 Minute nach der Injektion gewonnen wird, auf Antiserum negativ reagiert, reagiert es auch auf Sera negativ, welche innerhalb 7 Stunden nach Injektion mehreremal gewonnen werden, während sich positive Präzipitation durchaus nicht erhöht.

B. Experiment mit pflanzlichem Eiweiss.

Das Antiserum zur Präzipitation, welches ich gebrauchte, ist das Bohneneiweiss-Kaninchenimmunserum vom 3000 fachen Präzipitationstiter. Die Resultate meiner Versuche an Meerschweinchen, welchen ich Bohneneiweiss, das, nach Kjerdahlscher Methode gemessen, bestimmte Menge Eiweiss enthaltet, injizierte und wie oben behandelte, zeigt Tabelle VIII. Aber ich konnte dabei vorher feststellen, dass die Präzipitation des Serums gesunder Meerschweinchen gegen Kaninchenimmunserum negativ ist. Aus den Resultaten obiger Versuche lässt sich folgendes schliessen: wenn man Meerschweinchen betstimmte Menge pflanzlicher Eiweisslösung in Fussvene oder Pfortader injiziert und über Präzipitation der Sera untersucht, welche nach bestimmter Zeit aus Herzblut befreit werden, ist die Präzipitation der ersteren positiv, während die der letzteren negativ ist, obgleich die Injektionsmenge des Eiweisses dieselbe ist. Aber wenn man Eiweisslösung mehr als bestimmte Menge einspritzt, sind beide positiv, wenn es auch wie bei Versuchen mit tierischem Eiweiss grossen Unterschied zwischen den Reaktiosstärken gibt.

Also man kann rationell die Ursache der Ergebnisse dieser Versuche auf Lebertätigkeit zurückführen. Und durch Präzipitation kann man verstehen, dass die Leber der Meerschweinchen eine bestimmte Wirkung auf tierisches und pflanzliches Eiweiss ausübt.

2. Über Serumanaphylaxie durch Injektion in Pfortader.

Ich habe bereits in der Einleitung erwähnt, dass Uffenheimer und Awerbuch [16] diesen Versuch bei der Untersuchung über Anaphylaxie gemacht haben. Jetzt will ich unter Anwendung dieser Methode über Wirkungen der Leber auf Eiweiss als Antigen untersuchen und die Resultate oben beschriebener Versuche aufs neue feststellen.

A. Über Serumanaphylaxie durch Reinjektion in Pfortader.

Die Untersuchungsresultate der Autoren über das Wesen der Anaphylaxie sind noch nicht einig. Nach einer im allgemeinen für richtig zu haltenden Angabe wird Reaktionskörper nach einem bestimmten Zeitraum erzeugt, wenn das artfremde Eiweiss, d. h. Antigen zur Sensibilisierung in Körper eingespritzt wird. Und bei Reinjektion desselben Antigens tritt dieses mit Reaktionskörper in Verbindung, lässt starkgiftiges Anaphylatoxin entstehen und am Tier Anaphylaxie geschehen. Wenn aber ganzes Eiweiss oder ein Teil davon bei der Reinjektion am Einführungsgang vorbehalten wird, obgleich die Entstehung des Reaktionskörpers durch Sensibilisierung genug ist, bevor es mit dem Reaktionskörper in Verbindung tritt, soll die Anaphylaxie gar nicht oder nur wenig entstehen. Als ich an Meerschweinchen und Pferden unter denselben Bedingungen bei der Reinjektion dem einen in Pfortader und dem andern in Fussvene Experiment gemacht und über Unterschied zwischen beiden Präzipitationen untersuchte, bekam ich die Resultate. Achtzehn Meerschweinchen sind subkutan mit je 0.1 cc. Pferdeserum sensibilisiert. Nach vierzehn Tage eine Hälfte in Portader und andere in Fussvene bzw. je drei Gruppe in Menge von 0.1, 0.3 und 0.4 cc. reinjiziert. In Portadergruppe zwei Tiere von 0.1 cc. und einer von 0.3 cc. zeigten negativ. Einer von 0.1 cc. und je einer von 0.3 cc. und von 0.4 cc. leicht positiv, bloss zwei von 0.4 cc. typisch anaphylaktisch. Dagegen die in Fussvene reinjiziert worden sind, zeigten alle drei von 0.1 cc. leichte anaphylaktische Symptome und die übrige Tiere gingen ganz typisch positiv. Wenn die Reinjectionsmenge bei Serumanaphylaxie des Meerschweinchens eine bestimmte Grenze, d.h. der minimalen Dosis nahe ist, wiederholt sich die Injektion in Pfortader in Begleitung des kleineren anaphylaktischen Anfalls als bei der Injektion in Fussvene oder gar keines Anfalls. Je mehr die Reinjektionsmenge wird, desto gösser wird auch der Anfall, was endlich solche Folge hat, dass sich der Unterschied zwischen beiden nicht anerkennen lässt.

B. Über Serumanaphylaxie durch Injektion in Pfortader als Sensibilisierung.

Bei der Sensibilisierung der Serumpräzipitation spritzt man eine bestimmte Menge Pferdserum an den einen Meerschweinchen in Pfortader und an den anderen als Kontrollversuch in Fussvene ein und nach derselben Inkubationszeit reinjiziert man dieselbe Menge Serum in Fussvene. Wenn das Serumeiweiss bei Sensibilisierung in Pfortader ganz in der Leber fixiert wird, entsteht kein Reaktionskörper,

da es nicht in den Körperkreislauf übergeht. Folglich entsteht auch keine Anaphylaxie bei der Reinjektion. Ich glaube, dass die durch Reinjektion gleicher Menge erscheinende Anaphylaxie der beiden verschieden sind, da sich der Reaktionskörper nicht genug gestaltet, wenn sich ein Teil des Eiweisses als Antigens in der Leber fixiert. Die Resultate der wie oben ausgeführten Versuche führen, wie folgenden: als Vorbehandlung wurde je 0.01 cc. Normalpferdeserum fünfzehn Meerschweinchen in Portader und anderen fünfzehn in Fussvene injiziert. Zur Reinjektion braucht man dasselbe in Menge von 1.0, 0.8, 0.5, 0.3 und 0.1 cc. für je drei Stucke von ganzen vorbehandelten Tieren gleich in Fussvene. Davon je sechs Meerschweinchen, welche bei der Vorbehandlung 1.0 und 0.8 cc. Serum in Portader oder in Fussvene injiziert waren, zeigten ganz typisch anaphylaktische Symptome. Dann sind alle drei von Fussvenengruppe, dessen Sensibilisierungsmenge 0.5 cc. beträgt, ganz typisch positiv. Sowie einer von Portadergruppe ebenso typisch positiv, und zwei von derselbe schon negativ. Je drei von 0.3 und 0.1 cc. von Portadergruppe fielen ganz negativ aus. Dagegen gingen bei Fussvenengruppe zwei von 0.3 und einer von 0.1 gingen noch typisch anaphylaktisch an. Zur folgenden Anfassung: die Serumanaphylaxie hat im allgemeinen bei der Sensibilisierung in Pfortader leichteren Anfall als bei der in Fussvene und auch anaphylaktischer Tod geschieht weniger. Bei diesen Experimenten habe ich auch festgestellt, dass keine Veränderung an den Kontrollversuchstieren ergibt, indem ich Sensibilisierung oder Reinjektion physiologischer Kochsalzlösung derselben Menge in Pfortader ausführte. Indem ich an jedem Meerschweinchen Sektion ausführte, der einen anaphylaktischen Tod starb, konnte ich eigene bedeutende Lungenenphysem, verminderte Blutgerinnung und Hyperämie der Darmwand anerkennen. Daher stimmen die Resultate dieser Experimente mit denselben überein, welche Uffenheimer und Awerbuch angaben.

Aus den oben erwähnten Ausführungen kann man wissen, dass sich die Lebertätigkeit, das in Pfortader injizierte, artfremde Eiweiss gewissermassen zu fixieren und seine antigenöse Eigenschaft zu verändern, durch Präzipitation und Anaphylaxie erkennen lässt, wenn man gleich nicht sofort feststellen kann, ob das Wesen dieser Tätigkeit ihre Schutzwirkung oder Verdauung ist.

VII. Über die Veränderungen der Kohlenhydrate in der Leber.

Über die Wirkung der Leber auf Kohlenhydrate will ich hier nicht viel sagen, da viele Autoren seit Bernard und Hensen[25] genaue Untersuchungen darüber versucht haben. Bei der Untersuchung über Veränderungen des Eiweisses in der Leber wollte ich als Vergleichsversuch auch ihre Funktion auf Kohlenhydrate quantitativ versuchen. An dem einen Meerschweinchen gab ich eine bestimmte Menge Traubenzuckerlösung von bestimmter Konzentration (Von „Merk"-Gesellschaft) in Pfortader, nachdem ich sie regelmässig laparotomiert hatte, und den anderen spritzte ich in Fussvene ein. Nach Verlauf bestimmter Zeit entnahm

ich durch Herzpunktion etwas Blut, damit ich ihren Zuckergehalt nach Bangscher Mikromethode[18] mass. Aber es lässt sich leicht denken, dass sich der Blutzucker des Meerschweinchens durch Operation sehr leicht verändert. Unter Berücksichtigung dieser Hinsichten machte ich die Kontrollversuche. Wenn es Unterschied zwischen Mengen des Blutzuckers gibt, je nach die Injektionsstelle verschieden ist, sollen sie auf Lebertätigkeit auf Kohlenhydrate zurückgeführt werden. Die Resultate dieser Experimente zeigen, wie Tabelle IX lehrt, dass das Versuchstier, wenn man ihm eine bestimmte Menge Traubenzucker in Fussvene einspritzt, sofort an Hyperglycämie leidet und nach 3 Stunden wieder normal wird, während es nach 1 Stunde noch im Zustande der Hyperglycämie steht. Und bei dem Versuchstiere, dem man dieselbe Menge Traubenzucker in Pfortader injiziert, ist der Blutzuckergehalt immer kleiner als bei dem, welchem man sie in Fussvene einspritzt. Als ich dabei als Kontrollversuch den Traubenzuckergehalt des Meerscheinchens, dem man dieselbe Menge physiologischer Kochsalzlösung in Pfortader gab, und den desselben, den man nur laparotomierte, mass, erschien Hyperglycämie fast gleichgradig bei beiden. Und bei dem Tiere, dem man an der Haut leicht operierte, in Fussvene einzuspritzen, und demjenigen, welchem man dieselbe Menge Physiologischer Kochsalzlösung in Fussvene injizierte, sah ich keine Veränderungen an ihrem Blutzucker. Darum kann man rationell die Differenz auf die Lebertätigkeit auf Kohlenhydrate zurückführen, welche durch Abziehung der Differenz, die durch Abziehung des durch Laparotomie vermehrten Blutzuckers von dem durch Injektion in Pfortader vermehrten bleibt, von dem durch Injektion in Fussvene vermehrten entsteht. Wenn man Meerschweinchen vom 200 g Körpergewicht 1.0 ccm 20% Traubenzuckerlösung einspritzt, sieht man, dass sich Blutzuckergehalt nach 15 Minuten im Verhältnisse ca. 2 : 1 vermehrt, während das Verhältnis nach 1 Stunde ca. 4 : 1 ist, wie Tabelle IX zeigt. So steht die Lebertätigkeit auf Kohlenhydrate mit derselben auf Eiweiss fast im gleichen Zahlenverhältnisse an der Minimalgrenze, wo die Reaktion erscheint, wenn gleich die Versuchungsmethoden nicht gleich sind.

VIII. Schlussbetrachtungen.

1. Das Bohnenextrakt zeigt keine Präzipitation bei 100 facher Konzentration des Eiweisses gegen Sera der normalen Kaninchen und Meerschweinchen, wenn es streng neutral ist.

2. Das Bohnenextrakt-Antiserum zeigt Gruppenreaktion gegen Extrakt anderer Pflanzen derselben Familie, während es gegen das anderer Familie negativ ist. Nämlich diese Präzipitation hat eine Spezifizität.

3. Wenn dieses Extrakt mit Ammonsulfat vollgesättigt wird, entsteht eine Art Eiweiss. Dieses Eiweiss-Serum zeigt auch Gruppenpräzipitation.

4. Bei Immunisierung mit der ersten Fraktion und der zweiten, welche durch Halb- und Vollsattigung des Extraktes mit Ammonsulfat entstehen, bekommt

im allgemeinen die zweite hochgradigeres Antiserum als die erste. Und beide Antisera reagieren gegen beide Präzipitinogen positiv einander, aber jedes gibt gegen seinen eigenen Präzipitinogen hochgradige Reaktion.

5. Dass ein Teil des enteral eingeführten, tierischen oder pflanzlichen Eiweisses unverändert durch die Wand des Verdauungstraktus durchgeht und von ihr resorbiert wird, lässt sich sowohl bei Pflanzenfresser als bei Raubtier durch immunologische Reaktion, z. B. Präzipitation, Anaphylaxie anerkennen.

6. Dass ein Teil des Eiweisses, welches vom Pflanzenfresser oder Raubtier unter Normalfütterung aufgenommen wird, durch die Wand des Verdauungstraktus durchgeht, behaltend seine antigenöse Eigenschaft, von ihr resorbiert wird und in die Pfortader übergeht, wenn auch das Eiweiss seine antigenöse Eigenschaft in der Leber verliert, kann man durch Präzipitation wissen.

7. Man kann durch Präzipitation auch das nachweisen, dass das Eiweiss, wenn eine bestimmte Menge artfremden Eiweisses in Pfortader injiziert wird, weniger im Blut vorkommt, als bei dem Kontrolltier, welchem man dieselbe Menge artfremden Eiweisses in Fussvene einspritzt. Daher hat die Leber eine bestimmte Funktion auf artfremdes Eiweiss.

8. Die Anaphylaxie durch Injektion mit bestimmter Menge artfremden Eiweisses in Pfortader ist im allgemeinen leichter als dieselbe, welche durch Einspritzung derselben Menge in Fussvene erscheint. Daher hat die Leber eine bestimmte Tätigkeit auf artfremdes Eiweiss.

9. Als ich die Veränderungen der Kohlenhydrate in der Leber mit denjenigen des Eiweisses verglich, bemerkte ich, dass die beiden an der Minimumgrenze, wo die Reaktion erscheint, dieselben Zahlenverhältnisse haben, wenn gleich die Versuchsmethoden nicht gleich waren.

Die oben beschriebenen Schlussbetrachtungen will ich hier kurz gefasst schildern.

Wenn der Präzipitationstiter des Pflanzeneiweiss-Antiserums gleich im allgemeinen nicht so hoch ist, wie der des Tiereiweiss-Antiserums, hat das erstere immer eine Spezifizität gegen Pflanzeneiweissarten anderer Familie. Ein Teil des aufgenommenen, artfremden Eiweisses kann, seine antigenöse Eigenschaft, behaltend durch die Wand des Verdauungstraktus im Normalzustand durchgehen. Auf dieses resorbierte Eiweiss hat die Leber eine bestimmte Funktion, welche aber eine bestimmte Grenze hat. Wenn artfremdes Eiweiss über diese Grenze enteral eingeführt wird, geht ein Teil davon durch die Leber ins Blut unverändert über. Und wenn diese Manipulation wiederholt wird, so entsteht endlich der Antikörper. Die Menge des artfremden Eiweisses, welches durch die Wand des Verdauungstraktus unter Normalfütterung durchgeht, geht nicht über die Grenze dieser Lebertätigkeit. Das Aufnehmen des Eiweisses über eine bestimmte Menge ist darum physiologisch ungesund.

Aus den vorhergehenden Ausführungen will ich folgenden Schluss ziehen: die Assimilation des aufgenommenen, artfremden Eiweisses im Normalzustand hat

ihre Grenze in der Leber.

Zum Schluss erlaube ich mir, Herrn Vorstand Prof. Dr. M. Nagayo, und Herrn Abteilungsvorsteher Prof. Dr. K. Ishiwara für ihre freundliche Anregung und Anleitung zu dieser Arbeit meinen besten Dank auszusprechen.

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Tabelle I.

Meerschweinchen	Körpergewicht	Eingeführte Eiweissmenge	Ablauf nach Einführung	Resultate													
				Pfortaderblut					Lebervenenblut					Herzblut			
				unverd.	2f.	4f.	8f.	16f.	unverd.	2f.	4f.	8f.	16f.	unverd.	2f.	4f.	8f.	16f.
1	380 g	2f. 5 ccm	1 Std.	+	+	+	+	−	+	+	−	−	−	+	+	−	−	−
2	400	„	„	+	+	+	−	−	+	+	−	−	−	+	+	−	−	−
3	390	„	3 Stdn.	+	+	+	+	+	/	/	/	/	/	+	+	+	−	−
4	395	„	„	+	+	+	+	−	+	+	±	−	−	+	+	−	−	−
5	405	5f. 5 ccm	1 Std.	+	+	+	−	−	+	−	−	−	−	+	−	−	−	−
6	385	„	„	+	+	−	−	−	/	/	/	/	/	−	−	−	−	−
7	410	„	3 Stdn.	+	+	+	−	−	+	−	−	−	−	+	−	−	−	−
8	415	„	„	+	+	+	−	−	/	/	/	/	/	+	−	−	−	−
9	380	10f. 5 ccm	1 Std.	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
10	390	„	„	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11	375	„	3 Stdn.	+	+	−	−	−	±	−	−	−	−	−	−	−	−	−
12	380	„	„	+	−	−	−	−	/	/	/	/	/	−	−	−	−	−
13	405	20f. 5 ccm	1 Std.	−	−	−	−	−	/	/	/	/	/	−	−	−	−	−
14	390	„	„	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	370	„	3 Stdn.	−	−	−	−	−	/	/	/	/	/	−	−	−	−	−
16	395	„	„	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17	410	—	Vor Fütterung	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	375	—	Nach „	−	−	−	−	−	/	/	/	/	/	−	−	−	−	−
Kaninchen																		
1	1550	2f. 10 ccm	2 Stdn.	+	+	−	−	−	+	±	−	−	−	+	−	−	−	−
2	1600	„	„	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−
3	1450	5f. 10 ccm	„	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	1500	„	„	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−
5	1750	10f. 10 ccm	„	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	1700	„	„	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	1650	—	NachFütterung	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Tabelle II.

Meerschweinchen	Körpergewicht	Eingeführte Eiweissmenge	Ablauf nach Einführung	Resultate								
				Pfortaderblut			Lebervenenblut			Herzblut		
				unverd.	2f.	4f.	unverd.	2f.	4f.	unverd.	2f.	4f.
1	400 g.	100f. 15 ccm	1 Std.	+	+	−	−	−	−	−	−	−
2	385	„	„	+	+	−				−	−	−
3	390	„	3 Stdn.	+	+	+	±	−	−	−	−	−
4	320	„	„	+	+	±	−	−	−	−	−	−
5	375	10 ccm	1 Std.	+	−	−				−	−	−
6	380	„	„	−	−	−	−	−	−	−	−	−

7	370	10 ccm	3 Stdn.	+	–	–	–	–	–	–	–
8	320	„	„	+	–	–	–	–	–	–	–
9	350	5 ccm	1 Std.	–	–	–				–	–
10	340	„	„	–	–	–				–	–
11	350	„	3 Stdn.	–	–	–	–	–	–	–	–
12	375	„	„	–	–	–	–	–	–	–	–
13	360	–	Vor Fütterung	–	–	–				–	–
14	380	–	„	–	–	–	–	–	–	–	–
Kaninchen											
1	1650	100f. 30 ccm	2 Stdn.	+	+	–	–	–	–	–	–
2	1700	„	„	+	±	–	–	–	–	–	–
3	1800	10 ccm	„	–	–	–	–	–	–	–	–
4	1750	„	„	–	–	–	–	–	–	–	–
5	1800	–	Vor Fütterung	–	–	–	–	–	–	–	–

Tabelle III.

No.	Körpergewicht des Hundes	Eiweissarten	Eingeführte Eiweissmenge	Ablauf nach Einführung	Resultate: Pfortaderblut unverd.	2f.	4f.	8f.	16f.	Lebervenenblut unverd.	2f.	4f.	8f.	16f.	Herzblut unverd.	2f.	4f.	8f.	16f.
1	7500 g.	Eiereiweiss	100 ccm	1.5 Std.	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–
2	6800	„	60 ccm	„	+	+	+	+	–	+	+	–	–	–	+	+	–	–	–
3	6500	„	40	„	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
4	7200	„	100f. 300 ccm	„	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
5	6350	„	„	„	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
6	6550	„	Vor Fütterung	„	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Tabelle IV.

No.	K. d. K.	A. n. F.	Resultate: Pab.	Lvb.	Hl.
1	2750 g.	nach 1 Std.	+	–	–
2	2500	„	+	–	–
3	2650	„	+	–	–
4	2400	2 Stdn.	+	–	–
5	2570	„	+	–	–
6	2840	„	+	–	–
7	2450	5 Stdn.	–	–	–
8	2850	„	+	–	–
9	2700	„	–	–	–
10	2530	Vor F.	–	–	–
11	2650	„	–	–	–
12	2730	„	–	–	–

	K. d. M.				
1	250	nach 1 Std.	+	−	−
2	280	,,	+	−	−
3	360	,,	−		−
4	400	2 Stdn.	+	−	−
5	275	,,	+		−
6	370	,,	+	−	−
7	410	3 Stdn.	+	−	−
8	290	,,	−		−
9	320	,,	+	−	−
10	315	5 Stdn.	+	−	−
11	390	,,	−		−
12	395	,,	−		−
13	375	Vor F.	−	−	−
14	280	,,	−	−	−
15	290	,,	±		−
16	320	,,	−	−	−
17	295	,,	−	−	−

Tabelle V.

No.	Körpergewicht des Hundes	Ablauf nach Fütterung	Resultate		
			Pfortaderblut	Lebervenenblut	Herzblut
1	7550 g.	nach 1 Std.	+	−	−
2	6750	,,	+	−	−
3	6820	2 Stdn.	+	−	−
4	7830	,,	+	−	−
5	6770	3 Stdn.	+	−	−
6	6820	,,	+	−	−
7	5320	Vor Fütterung	−	−	−
8	5710	,,	−	−	−

Tabelle VI.

No.	Körpergewicht des Meerschweinchens	Injektionsmenge	Ablauf nach Injektion	Injektionsstelle	Resultate
1	250 g.	100f. 2.0 ccm	10 min	Fussvenen	+
2	260	,,	,,	,,	+
3	265	,,	,,	,,	+
4	250	,,	,,	Pfortader	+
5	270	,,	,,	,,	+
6	265	,,	,,	,,	+
7	270	100f. 1.5	,,	Fussvenen	+

8	260	100f. 1.0	10 min	Fussvenen	+
9	265	„	„	„	±
10	275	„	„	Pfortader	−
11	260	„	„	„	±
12	255	„	„	„	−
13	275	100f. 0.5	„	Fussvenen	+
14	260	„	„	„	+
15	255	„	„	„	+
16	260	„	„	Pfortader	−
17	250	„	„	„	−
18	270	„	„	„	−
19	255	100f. 0.2	„	Fussvenen	−
20	270	„	„	„	−
21	255	„	„	„	−
22	270	„	„	Pfortader	−
23	265	„	„	„	−
24	270	„	„	„	−

Tabelle VII.

Meerschweinchen	Injektionsmenge	Serumarten	Resultate 2f.	4f.	8f.	16f.	32f.	64f.	128f.
1	100f. 10 ccm	Fussvenen	+	+	+	+	−	−	−
2	„	„	+	+	+	+	±	−	−
3	„	„	+	+	+	+	−	−	−
4	„	Pfortader	+	+	−	−	−	−	−
5	„	„	+	+	±	−	−	−	−
6	„	„	+	+	−	−	−	−	−
7	100f. 0.5 ccm	Fussvenen	+	+	+	−	−	−	−
8	„	„	+	+	±	−	−	−	−
9	„	„	+	+	+	−	−	−	−
10	„	Pfortader	+	±	−	−	−	−	−
11	„	„	+	−	−	−	−	−	−
12	„	„	+	−	−	−	−	−	−

Tabelle VIII.

No.	Körpergewicht des Meerschweinchens	Injektionsmenge	Ablauf nach Injektion	Injektionsstelle	Resultate
1	370 g.	100f. 1.0 ccm	nach 10 Min.	Fussvenen	+
2	350	„	„	„	+
3	380	„	„	„	+
4	385	„	„	Pfortader	+

5	365	100f. 1.0 ccm	nach 10 Min.	Pfortader	+
6	370	,,	,,	,,	+
7	375	100f. 0.5	,,	Fussvenen	+
8	370	,,	,,	,,	+
9	360	,,	,,	,,	+
10	380	,,	,,	Pfortader	+
11	360	,,	,,	,,	+
12	375	,,	,,	,,	±
13	370	100f. 0.4	,,	Fussvenen	+
14	375	,,	,,	,,	+
15	380	,,	,,	,,	+
16	360	,,	,,	Pfortader	−
17	365	,,	,,	,,	±
18	385	,,	,,	,,	−
19	375	100f. 0.3	,,	Fussvenen	+
20	360	,,	,,	,,	+
21	365	,,	,,	,,	+
22	370	,,	,,	Pfortader	−
23	360	,,	,,	,,	−
24	365	,,	,,	,,	−
25	390	100f. 0.2	,,	Fussvenen	+
26	375	,,	,,	,,	+
27	380	,,	,,	,,	+
28	375	,,	,,	Pfortader	−
29	385	,,	,,	,,	−
30	380	,,	,,	,,	−
31	370	100f. 0.1	,,	Fussvenen	+
32	390	,,	,,	,,	±
33	380	,,	,,	,,	+
34	365	,,	,,	Pfortader	−
35	395	,,	,,	,,	−
36	380	,,	,,	,,	−
37	390	100f. 0.08	,,	Fussvenen	−
38	375	,,	,,	,,	−
39	380	,,	,,	,,	−
40	370	,,	,,	Pfortader	−
41	380	,,	,,	,,	−
42	395	,,	,,	,,	−

Tabelle IX.

No.	Körpergewicht des Meerschweinchens	Injektionsmenge	Injektionsstelle	Blutzuckerkonzentration. %			
				Vor Injektion	Nach Injektion 15 Min.	1 Std.	3 Stdn.
1	200 g.	20.0 Prozent Lö. 5.0 ccm Pro. Kilo. Körpergewicht	Fussvenen	0.11	0.28	0.17	0.10
2	210	„	„	0.10	0.27	0.18	0.11
3	205	„	„	1.11	0.29	0.17	0.09
4	200	„	„	0.11	0.28	0.18	0.11
5	205	„	„	0.10	0.29	0.19	0.10
6	200	„	Pfortader	0.11	0.25	0.15	0.11
7	210	„	„	0.10	0.24	0.15	0.10
8	195	„	„	0.12	0.25	0.14	0.09
9	200	„	„	0.10	0.26	0.16	0.10
10	195	„	„	0.11	0.25	0.15	0.10
11	205	Physiolog. Kochsalz-Lö. 5.0 ccm Pro. Kilo. Körpergewicht	Fussvenen	0.09	0.10	0.10	0.11
12	200	„	„	0.11	0.11	0.10	0.09
13	220	„	„	0.10	0.12	0.09	0.10
14	195	„	Pfortader	0.11	0.16	0.14	0.12
15	205	„	„	0.12	0.15	0.13	0.10
16	185	„	„	0.10	0.16	0.14	0.11
17	190	Laparatomie	—	0.11	0.15	0.13	0.11
18	210	„	—	0.10	0.16	0.14	0.12
19	208	„	—	0.11	0.15	0.14	0.10

Further Studies on Biological Activities of Serum Elements against Organ Toxins.

By

Dr. **Heinosuke Wago.**

(From the pathological Department of the Institute.
Head of the Department Prof. Mataro Nagayo.)

(Abstracted from Japanese originals in the Japanese Journal of Experimental Medicine Vol. V, No. 5 and Vol. VI, No. 8.)

Although, hitherto, accumulated investigations give evidence that normal sera display certain antidotical activities against organ toxins but knowledge with regard to the essential nature is still far from complete.

A large and important field, therefore, for observation and investigation of this nature invited my attention to undertake a further research of it.

In my following experiment, two kinds of toxins — human placenta toxin and mouse lung toxin — were employed on 38 rabbits (Oryctologus cuniculus Linneus) and 169 mice (Mus musculus var. albus) ; and my investigations have been carried out along the following lines.

(1) Estimation of the minimal lethal dose of organ toxins.

(2) Clinical and anatomical observations concerning the toxic actions of organ toxins.

(3) Influences of the placenta toxin treated with normal rabbit serum on normal rabbits.

(4) Influences of the placenta toxin on rabbits immunized with trypsin.

(5) Influences of the placenta toxin treated with trypsin immune rabbit serum on normal rabbits.

(6) Influences of the placenta toxin on rabbits treated with trypsin immune rabbit serum.

(7) Influences of the placenta toxin treated with trypsin immune rabbit serum kept for half a year in the ice-box on normal rabbits.

(8) Influences of the placenta toxin on rabbits treated with normal rabbit serum.

(9) Influences of the placenta toxin treated with inactivated normal serum or with inactivated trypsin immune rabbit serum on normal rabbits.

(10) Influences of the placenta toxin on rabbits treated with inactivated normal serum or with inactivated trypsin immune rabbit serum.

(11) Comparative studies on influences of the lung toxin treated respectively with activated or inactivated serum albumin of normal rabbits on mice.

(12) Comparative studies on influences of the lung toxin treated respectively with activated or inactivated serum globulin of normal rabbits on mice.
(13) Comparative studies on influences of the lung toxin treated respectively with activated or inactivated serum lipoids of normal rabbits on mice.
(14) Comparative studies on influences of the lung toxin treated respectively with activated or inactivated serum albumin, globulin and lipoids of trypsin immune rabbit serum on mice.
(15) Comparative studies on influences of the lung toxin treated respectively with non-heated or heated (for 30 minutes at 56°C) glucose salution (5%) on mice.

From these experiments the following results have been obtained.

(1) The toxicity of the organ toxins was almost coincident with many worker's observation; that is, in rabbits or mice, when introduced into the blood stream, the minimal lethal dose of the organ toxin gives rise anaphylactic symptoms such as trembling, uneasiness, difficulty in walking, dyspnea, and strong palpitation of the heart and sometimes to shock at a short time after the injection. As anatomical changes, there were produced some hypermia and hemorrhage through the body, a slight parenchymatous degeneration of the kidneys and liver, thrombosis in the lungs and liver, and an interference with blood coagulation.

(2) The placenta toxin treated with normal rabbit serum (organ toxin was mixed with serum in proportion to 2 and 1, and keeping for 30 minutes at 38°C) decreased its toxicity markedly when it was given in the minimal lethal dose or more, intravenously on normal rabbits.

(3) The rabbits immunized with trypsin have markedly shown an antidotical activity against the placenta toxin when it was given in the minimal lethal dose or more, intravenously.

(4) The placenta toxin treated with trypsin immune serum (the procedure has been done as in No. 2) did not show any toxic action on normal rabbits, when it was given in the minimal lethal dose or more intravenously.

(5) The rabbits treated with trypsin immune rabbit serum (the immune serum has been given intravenously in $\frac{1}{2}$ dose of the toxin at 20 minutes before) have markedly shown a great resistance against the placenta toxin, when it was given in the minimal lethal dose or more intravenously.

(6) The antidotical activity of trypsin immune serum against the placenta toxin seemed to have a relation on its antitryptic activity; and also the activity of the serum kept for half a year in the ice-chest remained, even though it was filtered through a Berkefeld candle, howerver, ist degree was very diminished.

(7) The rabbits treated with normal rabbit serum (the procedure has been done as in No. 5) have also shown an antidotical activity against the placenta toxin, when it was given in the minimal lethal dose or more intravenously, however, its degree was weaker than in the instance of trypsin immune serum. In these instances, the anatomical changes at one week after the injection showed some parenchymatous and fatty degeneration in the liver, kidneys and myocardium, the

growth of follicles and of histiocytes in the follicles of the spleen, and some times the growth of giant cells in the bone-marrow, and also some thrombosis in the lungs; however, there is not found any interference with blood coagulation.

(8) The placenta toxin treated with inactivated trypsin immune serum or with inactivated normal rabbit serum (the procedure has been done as in No. 2) killed normal rabbits in a few minutes when it was given in the minimal lethal dose intravenously, showing that the inactivated sera did not present any antidotical activity to the organ toxin.

(9) The rabbits treated with inactivated trypsin immune rabbit serum or with inactivated normal rabbitsserum (the procedure has been done as in No. 5) did not show any antidotical activity against the placenta toxin, when it was given in the minimal lethal dose intravenously. In these instances, the anatomical changes were almost coincident with the findings in No. 2.

(10) The antidotical activities of sera against organ toxins would perhaps not due to the hemolytic complement, although the activity was decreased or lost when sera were inactivated; because of that the antitryptic and antidotical activities were increased markedly in the instance of the immunization of trypsin, but not of the amount of hemolytic complement; and also that the antidotical activity of the serum kept for half a year in the ice-chest remained, even though it was filtered through a Berkefeld candle.

Considering the facts, therefore, the antidotical substance of sera against organ toxins and the hemolytic complement would not be identical.

(11) The lung toxin treated with serum albumin of normal rabbits (the organ toxin was mixed with serum albumin salt solution in proportion to 2 and 1, and keeping for 30 minutes at 38°C) decreased its toxicity markedly when it was given in the minimal lethal dose intravenously on mice, but not in the treatment with inactivated serum albumin.

(12) The lung toxin treated with serum globulin of normal rabbits (the procedure has been done as in No. 11) decreased its toxicity when it was given in the minimal lethal dose intravenously on mice, but not in the treatment with inactivated serum globulin; however, its degree was weaker than in the former.

(13) The lung toxin trated with serum lipoids of normal rabbits (the procedure has been done as in No. 11) showed a slight decrease of the toxicity when it was given in the minimal lethal dose intravenously on mice, but not in the treatment with inactivated serum lipoids.

(14) On the antidotical activity of those three elements of the serum against the lung toxin, albumin was the strongest and lipoids were the weakest among them.

(15) Such facts, as in Nos. 11, 12, 13, and 14, were almost the same in the instance of trypsin immune serum albumin, globulin and lipoids; and also the antidotical substances of sera are not dialytic.

(16) The lung toxin treated respectively with non-heated or heated (for 30 minutes at 56°C) glucose solution (5%) (the procedure has been done as in No. 11) showed

also a very slight decrease of the toxicity when it was given in the minimal lethal dose intravenously on mice, suggesting that blood sugar would also be acted more or less antidotically on organ toxins,

Summarizing these facts, there would be drawn the following conclusions, that is.

A. Rabbit serum has the antidotical activity against organ toxins such as human placenta toxin or mouse lung toxin in the rabbits or mice experiemented on, however, its activity was decreased markedly or lost when the serum was inactivated.

B. The antidotical substances would not be identical with the hemolytic complement, and also it is not dialytic.

C. On the antidotical activities of sera against organ toxins, the serum albumin seems chiefly to take the responsibility although the serum globulin has the activity in a slight degree ; and the serum lipoids and blood sugar also would be taken part in the activity in trace ; and moreover those seem to be raised in such instances as the antitryptic activity of serum would be raised in the immunization of trypsin. In short, on the antidotical activities of sera, there would be an important significance naturally in connection with each biological activity of the serum elements.

Result of the Prophylaxis of Weil's Disease (Spirochätosis icterohaemorrhagica) experimented both in Toyama and Chiba Prefectures.

By

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(From the fifth Section of the Bacterio-Serological Department.
Head of the Section: Prof. Kenzo Futaki)

The prevalence of Weil's disease has a close relation with our agriculture, and all the infected districts of this disease are paddy fields in valleys or along rivers and streams, marshy lowlands and quags around lakes and swamps, or boggy grounds near seashore. In those intensily infected places where many cases come about, there is an inevitable tendency among farmers that they dislike to pursue their work, of which fact I have already reported as the result of my investigation in Japanese reports, issued from Sanitary Department of Home Office, March, 17th 1919; ditto, December 11th, 1919, as well as in the Japanese journal of scientific Agricultural Society, No. 203, 1919.

I have at first experimented that the causal spirochaeta of Weil's disease (Spirochaeta icterohaemorrhagiae) lives and thrives well in water. Next I have studied and proven that spirochaeta, the cause of Weil's disease, increases immensely not only in the soil solution of Chiba, Ibaragi, Saitama, Niigata, Gifu, Shiga, Fukuoka, Saga, Kagoshima, Kohchi and some other prefectures where this disease is notably prevalent, but also in oozed solution of the alluvium as obtained in the eight representative large rivers in Japan such as the rivers Chikugo, Yoshino, Yodo, Kiso, Tone, Abukuma, Kitagami, Omono. The fact that the spirochaeta does live well in experimental paddy field has also been testified and published in Japanese journal of Experimental Medicine, Vol. IV, No. I, 1920 and the Japanese journal of the scientific Agricultural Society, No. 213, 1920, the above fact being fully ascertained, I determined to test whether we can prevent and exterminate the cause of this obnoxious disease by some improvement in manuring from our agricultural point of view. With this view I have made various experiments as to close relations between all kinds of fertilizers and spirochaeta, and at last found that calcium cyanamide is the unique remedy or fertilizer to this purpose. This fact being throughly and accurately ascertained in the test-tube, and next the

experiment was repeated in the small trial paddy field, and the same good results were obtained, of which I have reported already in the Japanese journal of the Government Institute for Infectious Disease Vol., II, No. I, 1918, as wellas in the Japanese journal of the scientific Agricultural Society, No. 189, 1918.

And furthermore by our trial ground experiment I have found out that spirochaeta thrives well in paddy fields and lives in such places where water and soil come in contact or the shallow part of the soil (published in Japanese journal of Experimental Medicine, Vol. IV, No. I, 1920; and the Japanese journal of the scientific Agricultural Society, No. 213, 1920).

Thus as I have been convinced of this fact evidently, I took the second step i.e. endevoured to make the field experiment practically and pursued it in 1920 at the most intensely infected locality, Tsukurimichi village, Imidzu district, Toyama prefecture, on the basis in such a way that the causal spirochaeta of this disease can possibly by disinfected by the application of calcium cyanamide at the rate of 5 "kwan" (18, 78 kilogramme) per "tan" (9.9173 are) with the good preventive effect as well as rich rice crop as already described in the Japanese journal of Experimental Medicine, Vol. V. No. I. 1921; and the Japanese journal of the scientific Agricultural Society, No. 224, 1921. Being encouraged by this satisfactory result, in the next year (1921) our experimental field in Toyama prefecture was extended over adjacent field as double in its area, while the same experiment was also conducted in some part of Chiba prefecture in the neighbourhood of Tokio. These detailed results were published in the Japanese journal of Experimental Medicine, Vol. VI, No. 8, 1922; as well as in the Japanese journal of scientific Agricultural Society, No. 240, 1922.

The brief statement of the results obtained in these three experimental fields in 1920 and 1921 is as follows: —

Part I First experiment in Toyama prefecture

1. Site of the experimental field; Tsukurimichi village, Imidzu district, Toyama prefecture; the experimental field is situated in the north part of Japan, but this locality is very fertile and its vicinity being noted for rice cultivation.

2. Area of the experimental field; 26 cho, 6 tan, 2 se, 10 bu, i.e. 26.407 hactare approximately.

3. Geographical feature of the experimental field; Swampy paddy field.

4. Soil of the experimental field; Alluvium loamy clay, rich in organic matter.

5. Farm-hands engaged in the experimental field; 236 in total, of which were 125 men and 111 women.

6. Number of the once infected with the Weil's disease; Among these 52 men and 28 women, 80 in total.

7. The most prevalent locality of Weil's disease was selected as the experimental field; This locality is known as the severest in virus in Japan and the average number of patients per year 1916 1919 is 26.

8. Duration of the experiment; From the beginning of May until the end of November 1920,

9. Number of day's work of our experimental field; 3389 men.

10. Quantity and period of calcium cyanamide used; 18.75 kilogramme of calcium canamide (16% of Nitrogen) per "tan" were applied at one time, from 5 th May to 18 th May 1920.

11. Other manures used in our experimental field; Besides calcium cyanamide soy bean cake, farm yard manure, fish scraps, calcium superphosphate, potassium sulphate, tobacco-ash and especially wood-ash and straw-ash were applied generally.

12. Method of checking of the result; For the convenience of examing of cases of Weil's disease we framed up a diary of all our farm-hands and recorded, whether they worked in the experimental field or outside of it. The growth and the yield of the crops were also examined.

13. Preventive result in the experimental field; A great many patients appeared in the neighbourhood of the experimental field, while no case we had in our experimental field.

14. Result of rice crops; Owing to the favourable weather in this year for paddy rice, we had an extraordinary successful in our experimental field compared with those of the adjoining fields. Average yield of rice crops of our experimental field and of Toyama prefecture in general is as follows: —

		Average yield per "tan"
(A)	In our experimental field (middle variety of rice: Nakate)	2.949 "koku" (53.082 litre)
(B)	Average yield of Toyama prefecture in 1919	2.127 "koku" (38.286 litre)
(C)	Average yield of these 5 years in Toyama prefecture	2.104 "koku" (37.872 litre)

The average yield of paddy rice in the experimental field, before we adopted it as trial ground, was roughly 2.5 "koku" (45.000 litre) in middle variety of rice per "tan" even in the favourable year. But it must be remenbered that the extraordinary good result we obtained in our experimental field, compared with those of adjacent fields owes evidently much to the improved way of manuring and not exclusively to the effect of calcium cyanamide.

Part II Second experiment in Toyama prefecture

Site of the experimental field, geographical feature of the experimental field, soil of the experimental field, duration of experiment and the method of checking of the result are the same to part I.

1. Area of the experimental field; 48 cho, 1 se, 17 bu, i.e. 47.6259 hactare approximately.

2. Farm-hands engaged in the experimental field; 462 in total, of which were 244 men and 218 women.

3. Number of the once infected with the Weil's disease; Among these

118 men and 73 women, 191 in total.

4. The most prevalent lacality of Weil's disease was selected as the experimental field. This locality is known as the severest in virus in Japan and the average number of patients per year 1916–1919 is 57.

5. Number of day's work of our experimental field; 6502 men.

6. Quantity and period of calcium cyanamide used; 18.75 kilogramme of calcium cyanamide (16% of Nitrogen) per "tan" were applied at one time from 6th May to 19th May 1921.

7. Other manures used in our experimental field; Besides calcium cyanamide, soy bean cake, farm yard manure, fish scraps, calcium superphosphate, potassium sulphate, tobacco ash, cement dust and especially wood and straw ashes were applied generally.

8. Preventive result in the experimental field; In this year especially a great many patients appeared in the neighbourhood of the experimental field, while we had but only one case in our experimental field.

9. Result of rice crop; Notwithstanding very unfavourable weather in this year for paddy rice than the preceeding year, rice cultivation of the experimental field was extraordinary successful on the whole compared with those of the adjoining fields. Average yield of rice crops of our experimental field and of Toyama prefecture in general is as follows:

		Average yield per "tan"
(A)	In our experimental field	
	1. Middle variety of rice (Nakata)	2.944 "koku" (52.992 litre)
	2. Late variety of rice (Okute)	3.219 "koku" (57.942 litre)
(B)	Averagr yield of Toyama prefecfure in 1920	2.135 "koku" (38.430 litre)
(C)	Average yield of these 5 years in Toyama prefecture	2.121 "koku" (38.178 litre)

The average yield of paddy rice in the experimental field, before we adopted it as trial ground, was roughly 2.5 "koku" (45.000 litre) in middle variety of rice and 2.8 "koku" (50.400 litre) in late variety of rice per "tan" in the favourable year. Referring to the extraordinary good result, we obtained in our experimental field, compared with those of adjacent fields, it owes evidently much to the improved way of manuring, and not exclusively to the effect of calium cyanamide.

Part III Experiment in Chiba prefecture

1. Site of the experimental field; Teradai, Narita town, Imba district, Chiba prefecture; the district where the experimental field lies, is situated in the environs of Tokio and the climate is very mild.

2. Area of the experimental field; 33 cho, 1 tan, 0 se, 2 bu, i.e. 32.0244 hactare approximately.

3. Geographical feature of the experimental field; Swampy paddy field.

4. Soil of the experimental field; Diluvium.

5. Farm-hands engaged in the experimental field; 120 in total, of which 58 men and 62 women.

6. Duration of the experiment; From the beginning of May until the end of November.

7. Number of day's work of our experimental field; 5383 men.

8. Quantity and period of calcium cyanamide used; 18.75 kilogramme of calcium cyanamide per "tan" (16% of Nitrogen) were applied from 20th May to 15th June 1921.

9. Other manures used in the experimental field; Besides calcium cyanamide, soy bean cake, night soil, calcium superphosphate and especially rough farm yard manure applied in general.

10. Method of checking of the result; Ditto in Toyama prefecture.

11. Preventive result in the experimental field; In this year a great many patients appeared in the neighbourhood of the experimental field, while no case we had in our experimental Teradai field.

12. Result of rice crop; Rice cultivation of the experimental field was successful on the whole compared with those of adjoining fields.

Average yield of rice crops of our experimental field and also of Chiba prefecture in general is as follows: —

		Average yield per "tan"
(A)	In our experimental field.	2.659 "koku" (47.862 litre)
(B)	Average yield of Chiba prefecture	2.165 "koku" (38.970 litre)
(C)	Average yield of these 5 years in Chiba prefecture	1.877 "koku" (33.786 litre)

The average yield of paddy rice in the experimental field before we adopted it as experimental field was roughly of 2.3 "koku" (41.40 litre) per "tan" even in the favourable year.

In conclusion I wish to express my appreciation to Professor Dr. M. Nagayo, Director of the government Institute for Infectious Disease and Professor Dr. H. Hayashi, the former Director, to Professor Dr. K. Futaki; to the Electric Chemical Industrial Co. which supplied me calcium cyanamide and to the authorities of both Toyama and Chiba prefectures as well as to the inhabitants of both the localities, who facilitated in every way.

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Experimental Studies on the Process of Formation of Vaccinal Immunity.

Especially on the Invasion of the Cutan Inserted Vaccine Virus into the Blood Circulation.

(The First Report)

By

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(From the first Section of the Bacterio-Serological Department, Head of the Section. Prof. Kikutaro Ishiwara.)

Contents.

Introduction.

Chapter I.

The method of Experiment.

1. General Principle
2. Animals employed for the Experiments
3. Vaccine Virus employed for the Experiments
4. The Method of Vaccination
5. The Method of obtaining Blood
6. The Method of Inoculation into the Testicle

Chapter II.

Testicular changes after the Inoculation of Blood or Organ-emulsion of Vaccinated Rabbits.

Chapter III.

Invasion in the Blood Circulation of Vaccine Virus Inoculated on the Skin Surface.

Chapter IV.

Relation of Pustule-formation to Invasion of Vaccine Virus into the Blood-Circulation.

Chapter V.

Fate of Vaccine Virus Injected into the Vein.

Chapter VI.

On question whether Vaccine Virus has Affinity to Skinny Tissues.

Chapter VII.

On Process of Formation of Vaccinal Immunity.

Chapter VIII.
Difference between Variola Virus and Vaccine Virus.
Chapter IX.
Paragraph I.
Experiment on the human body.
Paragraph II.
Relation between Blood at Different Periods and Scrotal Edema.
Paragraph III.
Time of appearance and continuation of vaccine virus in the Blood of Vaccinated Rabbits.
Summary
Literature.

Introduction.

Jenner's vaccination is the most typical pattern of all the active Immunization, the efficacy of which is universally acknowledged to-day. But how and by what cause is immunity effected, has been a question that remains ever unsolved. With all the hot arguments among the investigators, no satisfactory conclusion has, up to the present time, been reached with regard to the relation of the vaccine virus to the vaccinated organism. Some (e. g. Calmette et Guérin,[2] Hiller,[9] v. Prowazek,[26–28] Haaland,[8] Hauser,[7] Halberstädker, L., u. v. Prowazek, S,[10] Jürgens,[14] Mühlens u. Hartmann,[18] Nobl,[22] Paschen,[30] Ohly,[24] Süpfle,[35–37] and Camus,[4] insist that vaccine virus propagates only at the inoculated skin site, while others (e.g. Reiter, L. Pfeiffer,[25] Eichhorn, Frosch, Vanselow u. Freyer,[39] Neisser,[23] Siegel,[34] Wasielewski,[41–42] Aldershoff,[1] Casagrandi,[3] Mulas, Sion et Redulesco,[33] and Gins[5–6] maintain that it spreads over all parts of the vaccinated organism. This question is so important that the foundation of vaccinal immunity depends entirely on it. It carries a great importance not only in theory, but in every sphere of practice.

The methods of experiments to prove the vaccine virus which in the blood or organs of vaccinated organism which have widely been employed up till now, may be divided into two kinds. The first method is the provocation-method in which man vaccinates on the skin of the organism, then, excoriates some other part and examines if the excoriated site forms pustuls. The second is the transmission-method in which man inoculates on the skin or cornea with blood or

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organ emulsion obtained from some previously vaccinated organism, and examines the effect.

These methods of experiments, however, have proved to be wholly defective, because the results obtained by such methods have shown very few positive manifestations among the many experiments made by the investigators who are in favor of the affirmative opinion about the invasion of vaccine virus into the blood circulation. Moreover, vaccine virus has generally a very strong resistance against the influence of dryness, sunshine, frigidity, etc., and even the highly diluted vaccine virus can well form pustules. Such facts are greatly liable, in course of experiments, to the accidental infection of vaccine virus. Such accidental infection may be effected by the agency of flies, operators' hands, instruments, or stalls for examinated animals. During the time when the discussion on the distinction between variola virus and vaccine virus was very hot, the investigators belonging to L' Institut Superieur de la Vaccine made the following experiment with a view to disprove the arguments of the german investigators who had been maintaining uniquity. They scarificated the skin surface of some calves, and smearing desinfected glycerin on the scarification so made, and bandaging them up very carefully they kept these calves in well-disinfected stalls. After several days, they found vaccinal pustules growing on each of the calves. Mevius once had a barber shave a calf, but by some reason or other he postponed the inoculation for several days. After lapse of these days, he found a number of pustules on the calf's skin surface. These examples are also the grounds on which the investigators holding the opinions that the vaccine virus may not enter into the blood circulation-viz. non generalisation-attack those maintaining affirmative arguments.

It goes without saying, however, that it is improper to conclude merely from the arguments of the investigators maintaining that vaccine virus does not at all enter into the blood circulation. For, even in case of septicamia of protozoa or bacteria which we can prove morphologically or culturally, it is not always very easy to prove its agents satisfactorily in the blood or internal organs. So, it is, of course far more difficult to prove the vaccine virus in the blood or organs by dint of such imperfect methods as provocation or transmission, for, vaccine virus can not, in the present state of bacteriological development, be dealt with even morphologically or culturally. Accordingly, unless we have sufficient morphological knowledge or have discovered artificial cultivation of vaccine virus, we are compelled to employ some other method perfectly free from accidental infections of virus.

Taking these facts in view, I employed a method of cultivating the virus in the testicles of rabbits. This method had been formerly employed for other objects by Henseval and Convent, especially Noguchi,[21—22] and recently Kii, and his co-workers,[15] etc.

The testicles of rabbits give the following conveniences:

1. Large quantities may be injected at a time,

2. Injected virus may touch the testicular cells satisfactorily,

3. Accidental infections of vaccine virus through the air, etc. may perfectly be avoided, because the testicles are entirely covered with the scrotal skin,

5. The testicles afford something like elective medium, because, of their sentiveness and rapid multiplications as Noguchi emphasized in his work.

Employing the above mentioned method, I believe that I have reached the satisfactory conclusion regarding the relation of the vaccine virus to the vaccinated organism. In the forthcoming pages I am going to show the results of my experiments which have led me to the conclusion, and further to treat the questions regarding the theory of vaccinal immunity.

Chapter I.

The Method of Experiment.

1. General Principle.

In order to attain the object of my research, the following experiments were carried out. First I vaccinated normal rabbits with vaccine virus. Then a number of other normal rabbits were inoculated into each of their testicles with the blood or organ-emulsion obtained from the previously vaccinated rabbits. The testicles of these animals were examined every day if any change occurred in them. In case any of these testicles presented the expected changes, they were castrated one after the other to be tested successively. The testicles so castrated were made into an emulsion with sterile physiological salt or 60 per cent glyceric solution in the proportion of one gram of the testicle to 4.0 c.c.m. of the fluid, the emulsion was inoculated on the skin of other normal rabbits and calves to ascertain the existence of the vaccine virus in it. With the view of investigating immunologically, on the other hand, all the rabbits including both the castrated and non-castrated, were reinoculated on the skin surface or in the testicles with vaccine virus two-five weeks after the inoculation with the testifying materials,

Again, in order to examine the freeness of the testicles from all contaminating bacteria, the castrated testicles mentioned above were cultivated in the culture media (broth, agar, grape-sugar-agar, and blood-agar.)

By the method explained above, I could prove immunologically that the changes were caused by the vaccine virus, and the antivaccinal immunity was developed in the test animals.

2. Animals employed for the Experiments.

The young albino rabbits were mostly employed for the purpose. This was because of my supposition that the sensitiveness may characterize the testicles of Albino rabbits. As Voigt,[10] Süpfle,[38] and Sion et Randulesco[38] have pointed out, the skin of young rabbits are thinner and softer than those of grown-up ones; and, consequently, they are exceedingly sensitive and present very vivid demonstrations after vaccinations. But, of course, too young a rabbit accompanies various

inconveniences in course of experiments. Therefore, rabbits weighing from 1500 to 2000 gr. were mostly used for these experimental purposes.

3. Vaccine Virus employed for the Experiments.

The kind of vaccine virus used for the experiments were as follows: —

1. Variola-vaccine issued by our institute,
2. Variola-vaccine, cultivated in the rabbit testicles,
3. Variola-vaccine cultivated exclusively on the calf skin,
4. Vaccine lymph produced by Kitasato Institute.

In order to testify their virulence 0.5 c.c. of various kinds of diluted vaccine virus were inoculated into rabbits testicles, and skin was inoculated by Chalmette and Guérin's method. The following tables demonstrate the results

Table I.

Tests of Virulence of Variola Vaccine 33. G. (Kawasaki) Cultivaled in the Rabbits Testicle.

Degree of Dilution	Rabbits		Autopsy
	Skin	Testicle	
10.0	##	1 dy: Already edema and induration, then increased	Edematous hemorrhageous form
100.0	##	1 dy: Already slight edema and induration, then increased	Edematous hemorrhageous form
500.0	##	3 dys: Slight edema and induration 5 dys: Increased	Mixed form
1000.0	##	3 dys: Slight edema and induration 5 dys: Increased	Mixed form
5000.0	##	3 dys: Slight edema and induration 6 dys: Slight edema and marked induration.	Mixed form
10000.0	±	3 dys: Very slight edema and slight induration 5 dys: Slight edema and marked induration	Granular form
50000.0	—	6 dys: No edema but induration 7 dys: Increased	Edematous hemorrhageous form
100000.0	—	6 dys: No edema but induration	Slight form
200000.0	—	7 dys: No edema but induration	Slight form
500000.0	—	No change	Normal

Note: ## indicates the coufluent pustules # indicates the formation of partly confluent, partly disseminated Pustules, + indicates the formation of disseminated pustules, ± indicates the formation of few pustules, — indicates the no pustule formation

Table II.

Test of Virulence of Variola vaccine 15 g. cultivaled on the Calfskin.

Degree of Dilution	Rabbits		Autopsy
	Skin	Testicle	
10	##	1 dy: Already edema and induration, the increased	Edematous hemorrhagic form
100	##	1 dy: Slight edema 2 dys: Edema and induration 4 dys: Increased	Edematous hemorrhagic form
500	##	2 dys: Slightly indurated 3 dys: Edema and induration 4 dys: Increased	Mixed form
1000	##	3 dys: Slight edema and induration 5 dys: Increased	Edematous hemorrhagic form
2000	+	3 dys: Edema and slight induration 5 dys: Increased	Edematous hemorrhagic form
5000	—	3 dys: Induration 5 dys: Increased	Mixed form
10000	—	2 dys: Very slight induration 3 dys: No edema but induration	Slight form
50000	—	3 dys: Slight induration 5 dys: No edema but induration	Slight form
100000	—	No change	Normal
200000	—	No change	Normal

Note: The signs are the same signification as in the preceding table.

4. The method of vaccination.

Back skin or ear tip were shaved and washed, and immediately after that, vaccine virus was inoculated by a disinfected knife. Sometimes I employed the methods of cross- or pickinoculation. Direct injection into the veins was also done in some special cases.

5. The method of obtaining Blood.

Blood for the use of experiments was taken from the heart or ear-vein. In the former case, their breast-skin was shaved, disinfected by alcohol and tincture of iodine, and by means of syringe blood was cautiously sucked out from the heart. In the latter case, care was taken to puncture the veins of the ear not inoculated previously. To prevent the coagulation of blood, 4% dilution of citrate of natrium was mixed, in the ratio of 0.1 c.c.m. of it against 1 c.c.m. of blood.

6. The method of Inoculation into the Testicle.

Rabbits were kept laid on their back, and their scrotal skin were disinfected with alcohol and tincture of iodine. Then their testicles were pressed in the scrotum by the left hand of the operator, and by the other hand needle of a syringe was thrust into the testicular parenchyma along the long axis. The point of the needle is prevented from passing through the parenchyma to the tunica vaginalis. The contents are now forced out of the syringe into the organ at different regions by turning the direction of the needle.

Chapter II.

Testicular Changes after the Inoculation of Blood or Organ-emulsion of Vaccinated Rabbits.

It goes without saying that the testicular changes after the inoculation of vaccine virus in the testicles may vary according to the degree of sensitiveness of the inoculated organism. But it is none the less true that, as Noguchi's[21] experiments and mine demonstrate plainly, the changes may come out more noticeably as the virulence or concentration of the vaccine virus increases. (See Table I and II.)

High virulent and concentrated Vaccine virus shorten the incubation, that is, in other words, reaction is brought about quickly as well as intensely. On the contrary, vaccine virus of low virulence and high dilution lengthen the period of incubation and the reaction is weak.

The testicular changes caused by the injection of blood or organ-emulsion containing vaccine virus, may be divided into the following two periods. (1) Period of Incubation, and (2) Period of Swelling. It is of course provided in this case, however, that there are, as explained in the last chapter, some testicles which present practically no sign of reaction.

(1) Period of Incubation.

It takes about 1–9 days after inoculation before the testicles show the proper signs of vaccine virus. The following sub-divisions may be made in consideration of the kinds of blood employed for injection.

(a) Blood of initial period, i.e., blood obtained from the rabbit at the end of 1–3 days after vaccination, was injected into rabbit's testicles. This injection produced on the following day edema and slignt congestion on the scrotal skin. Changes which followed varied in their demonstrations, according to the differences of the sensitiveness of the testicles. The edema disappeared, in the some cases, very rapidly; and in some other cases, very gradually, and in the rest, showed practically no change of any kind. The testicular parenchyma was slightly swollen and indurated on the following day. But the swelling and induration disappeared in a few days.

(b) Blood of acme period, i.e., blood obtained from rabbit 4-6 days after vaccination, produced on injection, no special changes in the testicles. Only I

could observe very slight ecchymosis or edema which rapidly disappeared from the scrotal skin. The testicular parencyma became moderately indurated, but the symptoms disappeared quickly.

(c) Blood of terminal period, i.e., blood obtained from rabbit at the expiration of 7–9 days, was injected. In this case, the testicles became markedly edematous on the following day, generally speaking, more than in the case of (a). The parenchyma was only slightly indurated, though the scrotal skin was edematous very markedly.

(d) Blood of late period, i.e., blood obtained from rabbit more than 9 days after vaccination, was injected into the testicular parenchyma. The injection produced at each of the inoculated sites on the scrotal skin spots of red color of about the size of finger-tips. These red spots disappeared in a few days, and the moderate induration and swelling which had been observed in the testicular parenchyma also disappeared rapidly. Also, it must be noted that some testicles presented no change whatever.

(2) Period of Swelling.

The testicles injected with the blood of initial and acme period presented induration and swelling in the parenchyma. The change on the scrotal skin was entirely absent. I call this phenomenon "*preswelling*". On the following day the swelling and induration of the testicle increased rapidly, and the testicle became unable to pass through the inguinal canal. Even a slight touch of finger on the part affected seemed terribly painful to the animal. The indurated testicular parenchyma was felt something like cartilagineous matter. The scrotal skin showed congestion and hemorrhages. Vesicles containing transparent fluid of yellowish red color were in some cases observed on the scrotal skin. The testicle presented dark purple color, and its size became as large as the egg of pigeons or hens. The penis was also congested, indurated and showed bleeding spots; the color turned into dark red. The prepuce became so indurated and swollen that it became any longer unable to keep its proper position.

The testicles injected with blood of terminal period (c) mostly presented changes similar to the above mentioned changes but, in this case, however, no marked induration was observed after the disappearance of edema, but only a few small nodes scarcely recognizable by palpation.

Anatomical Changes.

The following statements are the results of my anatomical investigations of the scrotal and testicular changes. The investigations were made by naked-eyes.

The scrotum becomes severely edematous, and adheres closely to the tunica vaginalis. Its color turn into dark purplish red, and marked congestion or hemorrhages are observed on the scrotal skin. The tunica vaginalis also becomes

severely congested and edematous. In most cases transparent yellowish red exsudative fluid is found in the part where the scrotum and the tunica vaginalis adhere to each other. The testicle also adhere to the scrotum and shows a grey fibrous material sticking in the surface.

Changes of the testicular parenchyma may be divided into two leading types : (1) Severe form, and (2) Slight form.

(1) Severe form.

This type of change is observed in the majority of the testicles injected with blood of initial, acme and terminal period. The testicular parenchyma becomes highly swollen. Induration, edema, congestion and hemorrhages are all severe. The color of the testicle becomes dark red, spotted here and there with yellowish grey granula in the size of millet or pinhead. These granula are frail. Close investigation in the severe form gives the following subdivisions of the type.

(a) Edematous hemorrhageous form.

In this type the testicle is covered all over with numerous hemorrhagic area, and the edema and hemorrhages are so severe that the aspect of normal testicular parencyma is all gone. On the section of testicular Parenchyma is recognized exudation of a large quantity of serous fluid, which has been produced in the course of severe edematous change. Though the swelling is marked, induration is not so marked in this type, and the yellowish grey granula are scarce.

(b) Granular form.

This type present numerous granula in the testicular parenchyma. The tissue is highly congested and frail and spotted here and there with small hemorrhagic areas. Although the degree of swelling is not so marked as the edematous-hemorrhagic form, but induration is very severe.

(c) Mixed form.

This type stands between the edematous-hemorrhagic and the granular form.

(2) Slight form.

This type is found very rarely among the testicular parenchyma injected with terminal blood. The section presents a few yellowish grey granula around which congestions and hemorrhages are observed. Induration and swelling are both moderate in this type and the organ is not so frail.

Chapter III.

Invasion in the Blood Circulation of Vaccine Virus Inoculated on the Skin Surface.

Experiment I.

Rabbit No. I. (weight 2100 grs.)

Nov. 24, 1919. The back skin was shaved and inoculated on an area

covering 5 × 6 sq. cm. with a 1 : 1000 dilution of variola vaccine (*33rd generation*) of rabbit testicular cultivation. On Nov. 26, on the inoculated skin-surface produced a slightly elevated papules. On Nov. 28, these papules increased in size and density until most of them united together isolating only a small part. The papules formed on their heads crusts of reddish black color around which was tinged with red and raised from reddish black crust. On Nov. 30, the papules became markedly dry, and from that day the papules tended to healing. Dec. 10, the papules recovered leaving only slight scar. Dec. 20, the back skin (5 × 8 sq.cm) and the testicles (0.5 c.c.m.) were reinoculated with variola vaccine (*33rd generation*) (of rabbit testicular cultivation). The back skin presented no sign of vaccinal pustules, and the testicles also showed no vaccinal change whatever.

The heart of the animal was punctured and blood was taken out to be tested with the view of ascertaining the existence of vaccine virus by injecting the blood into the testicles of other rabbits. This test was repeated for nineteen days from the eighth hour after vaccination. The following table demonstrates the results of the experiment:

Table III.

Rabbit		Date of injection		Injected material and method of injection	Testicular changes after the inoculation	Castrated Result of inoculation on skin testicles with resp. testicular emulsion				Termination	Date of revaccination and its result
Number	Weight					Autopsy	Bacteria	Rabbits	Calves		
2	1900	Nov. 24.	8 hours		No change besides ecchymosis	Normal	(—)	—	—	Dec. 17. †	
3	1730	Nov. 25.	1 dy.		1 dy: Both testicles edema and slight induration which increased 5 dys: tended to recovery 5 dys: Left testicle castrated	Slight form	(—)	+	+	living	Dec. 20. Skin — R. Testicles +
4	2650	Nov. 26.	2 dys.		1 dy: Both T. slight edema and induration which all gone 6 dys: Right T. induration 7 dys: Increased 8 dys: L. T. no change 9 dys: R. T. castrated	Edema as hemorrhagic form	(—)	+	+	living	Dec. 20. Skin — L.T. —
5	2100	Nov. 27.	3 dys.		3 dys: R. T. slight edema and induration which increased 5 dys: L. T. castrated 23 dys: R. T. castrated	L. edematous hemorrhagic form	(—)	L. + R —	L + R —	living	Dec. 20. Skin —
6	1750	Nov. 24.	4 dys.		3 dys: R. T. edema and induration 5 dys: Increased	L. edematous hemorrhagic form	(—)	+	+	Dec. 5 †	

8	2000	Nov. 29.	5 dys.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm of which injected into each of the testicles	3 dys: L. T. edema and induration then increased 6 dys: R. T. edema and induration 6 dys: B. Ts. castrated	Granular form	(−)	+	+	Dec. 6. †	
9	2310	Nov. 30.	6 dys.		5 dys: Slight induration 8 dys: Normal	Normal	(−)			Dec. 14. †	
10	1650	Dec. 1.	7 dys.		1 dys: Severe edema on both testicles 3 dys: Edema disappeared 4 dys: Edema and induration then increased 5 dys: B. Ts. castrated	Edematous hemorrhag form	(−)	+	+	Dec. 10. †	
11	1500	Dec. 2.	8 dys.		1 dy: B. Ts. marked edema 3 dys: Edema disappeared 5 dys: Edema and induration 6 dys: Increased 9 dys: B. Ts. castrated	Granular form	(−)	+	+	Dec. 13. †	
12	1750	Dec. 3.	9 dys.		1 dy: B. Ts. severe edema and slight induration 3 dys: Edema disappeared 7 dys: nodes in the R. T. but L. T. normal 7 dys: R. T. castrated	Slight form	(−)	+	+	Dec. 15. †	
13	1580	Dec. 4.	10 dys.		1 dy: B. Ts. Edema and induration 2 dys: Normal 7 dys: B. Ts. Edema and induration 9 dys: R. T. Increased, L. T. recovered 14 dys: Castrated	Edematous hemorrhag form	(−)	+	+	living	Dec. 26. Skin —
16	1920	Dec. 5.	11 dys.		6 dys: L. T. induration and on the following day normal					living	Dec. 20. Skin — B. Ts. —
19	1350	Dec. 7.	13 dys.		2 dys: B. Ts. Erythema 7 dys: Erythema disappeared gradually					living	Dec. 20. Skin — B. Ts. —
22	2500	Dec. 9.	15 dys.		No change					living	Jan. 4. Skin — B. Ts. —
27	1700	Dec. 11.	17 dys.		No change					Jan. 7. †	
31	1830	Dec. 13.	19 dys.		No change					living	Dec. 26. Skin + B. Ts. +

Note: In this and all subsequent tables, the use of the same signs is observed with the following signification:

+ indicates the vaccinal changes. ± indicates the vague changes. − indicates the no changes. × indicates the result unknown because of early death. (+) indicates the bacteria on the bacteriological test. (−) indicates the no bacteria on the bacteriological test. R. indicates the right testicle. L. indicates the left testicle. C. indicates the control test.

As the above table demonstrates clearly the results of Experiment, I show that vaccine virus, if inoculated on comparatively large areas, enters into blood already on the next day of vaccination.

Except Rabbit No. 9, all the rabbits' testicles injected with the blood from 2 to 10 days after vaccination presented, after the incubation period covering 3–7 days, the typical vaccinal changes.

Some other rabbits and calves were inoculated on the skin with the testicular emulsion prepared from these testicles, which formed perfect pustules. Moreover, the effect of revaccination on these rabbits was all negative. These results lead us to the conclusion that virus actually existed in the blood obtained from the heart of the vaccinated rabbit. Again, with regard to the fact that the testicles injected with the blood taken out from the vaccinated rabbit's heart on the eleventh, thirteenth and fifteenth day after vaccination, presented no marked change, but the revaccination could not effect any vaccinal changes, it is reasonable to conclude that very small quantity of vaccine virus still existed in the blood. It is of course absurd to think that only 2 c.c.m. of blood can bring about passive immunity, since the injection with a big quantity of blood after vaccination or convalescent of small pox can not easily effect passive immunity. Süpfle[35] maintains that some rabbits do not react against the vaccination. But this assertion of Süpfle can not explain the negative result which the revaccination presented in the above cases.

The testicular emulsion prepared (on the 5 days after the injection of blood) from the left testicle of rabbit No. 5 which had been injected with the blood of the third day, produced marked pustules on the rabbits' and calves skin, but the emulsion prepared (on the 23 days after the injection of blood) from the right testicle of the same rabbit produced no pustules. This fact is due to the vaccinal immunity of the rabbit which had been complete: and, consequently, the virus had been absolutely exterminated.

Concluding from the above statements, I can safely assert that, in the body of rabbit No. 1, vaccine virus had been circulating with blood until the fifteenth day from the next day of vaccination. The testicles of rabbit which were injected with the blood obtained from the vaccinated rabbit on the following day of vaccination, showed typical changes in its testicles. The testicular emulsion prepared from the left testicle of this rabbit, was inoculated on the rabbit's and calves' skin which presented some vaccinal pustules.

The question which arises is what is the cause that brought about marked induration and swelling of the right testicle by the reinoculation in the same, while the complete vaccinal immunity was clearly detected on the skin-surface. Vaccinal immunity, according to the principle which is now considered as established, is effected only on the skin surface, and has nothing to do with the internal organs. But in many other instances as explained later on, vaccinal immunity is effected even in the testicles as well as on the skin surface. Thus I am inclined to think whether the above question can been explained by allergy. With regard to this question, however, we must wait for the results of further researches in this field.

By the experiments explained above I must come to the conclusion that the vaccine virus inoculated on the skin surface enters into the blood-circulation. But

several important questions still remain unanswered. The question are, whether the invasion is due (1) to the inoculated area being comparatively large, (2) to the individual difference of the rabbits employed for the purpose, or (3) to the virulence of the vaccine lymph.

The following experiment were all elaborated with a view to answere these question.

As to the third question, it may well be imagined that vaccine virus will enter into blood, since it happen though very rarely that the human body which is inoculated with young variola vaccine in the first or second generation of variola virus is generalized all over the body. But in the case of my experiments, the question is unreasonable, because the vaccine virus employed for the purpose was variola vaccine in the thirty-third generation of rabbit testicular cultivation; thus making it impossible to say that it had not been sufficiently vaccinalized.

Experiment II.

Rabbit No. 14 (weight 1620 grs.)

Dec. 5. 1919, the back skin was shaved and inoculated on an area covering 2 c.m. square with a 1 : 200 dilution of variola vaccine in the thirty-third generation of rabbit testicular cultivation. Dec. 8, slightly raised confluent papules of dark crimson color was observed, which became gradually dry, and on Dec. 10, part of papules was found dark red, around which was tinged with crimson color. Dec. 12, the rabbit died. From the next day of vaccination to the previous day of death, the heart was punctured, and blood was taken out every day, which was tested carefully by injecting with it into the testicles of rabbits.

The results are summarized in the following table:

Table IV.

Rabbit		Date of injection		Injected material and method of injection	Vaccinal changes after the Inoculation				Termination	Date of revaccination and its result	Result of inoculation on skin with resp. testicular emulsion	
							Testicles					
Number	Weight				Cornea	Skin	Induration edema etc.	Contaminating bacteria			Rabbits	Calves
17	1390	Nov. 6.	1 dy.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1ccm. of which was inoculated into each of the testicle: 0.7 ccm. on the skin, 0.3 ccm. on the cornea	−	− (−)	−		Nov. 18. †			
20	2030	Nov. 8.	3 dys.		−	+ (+)	+	(−)	Nov. 18. †		+	+
24	1700	Nov. 9.	4 dys.		−	− (−)	+	(−)	Nov. 18. †		+	+

25	2100	Nov. 10.	5 dys.	−	$\frac{+}{c+}$	+	(−)	Nov. 18. †	+	+
28	1650	Nov. 11.	6 dys.	−	$\frac{-}{c-}$	−	(−)	Nov. 19. †	+	+

Table IV indicates, as it was in the case of Experiment I, the existence of vaccine virus in the vaccinated rabbit's blood taken out on each of the third, fourth, fifth and sixth days. The question whether the invasion of vaccine virus into the blood is the result of individual difference of the rabbit employed for the purpose, has now been answered conclusively in the negative. Thus, the preceding two experiments have made it unquestionable that the vaccine virus undoubtedly existed at least for a certain period of time in the blood-circulation of vaccinated rabbit's body, only provided that comparatively large area of the skin was inoculated. In the case of human body, vaccination is done at a very small site. Then can vaccine virus still invade into the blood-circulation even in such a case? To answer this question I inoculated vaccine virus on the nasolabial part of a rabbit with a lancet, and investigated the existence of vaccine virus in the blood of rabbit's body.

Experiment III.

Rabbit No. 15 (weight, 1450 grs.)

Dec. 5. 1919. One insertion was made on the skin of the nasolabial part with the variola vaccine in the thirty-third generation of rabbit testicular cultivation. Dec. 7, a crimson area was observed around the inserted site. Dec. 9, the area changed into a papule, which, on the following day, turned into pustule with depressed head and marked areola. Dec. 12, the pustule was erosed. Dec. 14, the pustule was highly dry and on its surface dirty crust of dark grey color was detected. Dec. 21, the rabbit died from some unknown cause. Up to the twelfth day from the next day of inoculation, blood was taken out every day to be tested with the view of ascertaining the existence of vaccine virus by injecting with it the testicles of other rabbits, as shown in the following table, which is the summarized result of my experiments.

Thus, the rabbit No. 15 of Experiment III, though it was inoculated on a minimum site, vaccine virus was unquestionably proved to have been contained in the blood during the period covering from the third to the nineth day after inoculation.

In the preceding two experiments (Experiment II and Experiment III), the skin and cornea of the rabbits were simultaneously inoculated, when their testicles were inoculated with the blood of the vaccinated rabbits. Comparison was made among the results so obtained from these three different sites of inoculation. The

Table V.

Rabbit		Date of injection		Injected material and method of injection	Vaccinal changes after the inoculation				Termination	Date of revaccination and its result	Result of inoculation on skin with resp. testicular emulsion	
							Testicles					
Number	Weight				Cornea	Skin	Induration edema etc.	Contaminating bacteria			Rabbits	Calves
18	1900	Nov. 6.	1 dy.	3 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles; 0.7 ccm on the skin, 0.3 ccm. on the cornea	−	C −	−	|	living	Dec. 20. skin + testicles +	|	|
21	1790	Nov. 8.	3 dys.		−	C −	+	|	living	Dec. 20. skin − testicles +	|	|
23	1570	Nov. 9.	4 dys.		−	C +	+	(−)	Dec. 19. slaughtered	|	+	+
26	1370	Nov. 10.	5 dys.		−	C +	+	(−)	Dec. 24. slaughtered	|	+	+
29	1800	Nov. 11.	6 dys.		−	C +	+	(−)	Dec. 21. †	|	+	+
30	2100	Nov. 12.	7 dys.		−	C −	+	(−)	living	Dec. 26. skin − testicles −	+	+
32	2050	Nov. 13.	8 dys.		−	C −	+	(−)	Dec. 22. slaughtered	|	+	+
33	1350	Nov. 14.	9 dys.		−	C −	±	|	living	Dec. 26. skin − testicles −	|	|
36	2030	Nov. 15.	10 dys.		−	C −	−	|	living	Dec. 26. skin + testicles −	|	|
43	2700	Nov. 17.	12 dys.		−	C −	|	|	Dec. 21. †	|	−	−

cornea in all cases presented no vaccinal changes, while the skin of rabbit No. 20 and 25 reacted typically which had been inoculated with the blood obtained from the rabbit No. 14 on the third and the fifth day after the vaccination. The rabbits (No. 23, 26 and 29) which had been inoculated with the blood of the rabbit No. 15 (on the forth, the fifth and the sixth day), also showed vaccinal reaction, though it was very weak and slight. Meanwhile, the skin which had been shaved for the purpose of control test and not inoculated, presented papules hardly

distinguishable from that on the inoculated site. Small quantity of the papules was scratched off from each of the two sites, and two kinds of glyceric emulsion were prepared from it. The emulsion so prepared was inoculated respectively on rabbits' and calves' skin surface, which showed pustules in both emulsion. Consequently, the following three conclusions might be thought as possible. *Firstly*, both the inoculated and control sites produced pustules by the cause from outside, by means of accidental infection; *secondly*, vaccine virus which had been contained in the blood was transferred to the control site through the agency of the animal's tongue; *thirdly*, vaccine virus which entered into the blood-circulation from the testicle formed pustules on the injured site on the shaved skin surface. It is of course exceedingly difficult to decide which conclusion is right. At least, however, the third conclusion may not be right, because the skin of some other rabbits showed no vaccinal changes, while the testicles of most of the rabbits in a typical manner. As to the first and second conclusions, I should like to assert that the second is more appropriate in this case, for the testicle which had been injected with the same blood presented marked vaccinal changes. And, many investigators, who employed transmission method on the skin or cornea, have also obtained the positive results in these periods. In short, no satisfactory conclusion was reached in this comparative experiment.

Variola vaccine employed in each of the preceding experiments was the virus which had been cultivated in the rabbit testicle and had been transferred again and again form the testicle of one rabbit to that of another. Vaccine virus cultivated in such a way may naturally be very effective in its virulence to the rabbit's body. Here arises the question whether the invasion of virus is possible even if some other kind of vaccine virus be used for vaccination.

With the object of solving this question experiments were repeated in the following method, namely, to employ the vaccine virus which had never been cultivated in the rabbit body.

Experiment IV.

Rabbit No. 91 (weight 1830 grs.)

Jan. 21, 1920. One insertion was made on the skin of the nasolabial part by means of stab-inoculation. For the inoculation, I employed variola vaccine in the fifteenth generation on the calf skin. The original and the second generation of the virus were in the body of two monkeys. No change was detected on the following day. Jan. 23, markedly raised papule was observed on the surface. Jan. 24, the head of papule turned into pustule, which gradually increased in size and density. Jan. 25, depression was observed on the head. The pustule was surrounded with marked areola. Comparatively dry. Feb. 2, the pustule was entirely covered with crust. On the same day, in course of the heart puncture, the rabbit died from internal hemorrhage. From the second day after

vaccination to the day of death, blood was obtained every twenty-four hours and was injected into the testicles of rabbits to be testified its containing of vaccine virus.

In the experiments described above, not only the kind of vaccine virus was changed, but also the blood was obtained from the ear-vein instead from heart. This was because of the fact that the blood obtained from the peripheral vein had not been employed in the previous experiments.

The reason why the heart blood, and not the peripheral vein blood, had always been employed in the previous experiments is due to my imagination that, vaccine virus might possibly precipitate on the skinny tissues, when passing through the capillaries, because many investigators insist that vaccine virus has a affinity exclusively to the skinny tissues. As has been described preceding pages vaccine virus was well detected in the heart blood; and, therefore, in the following experiments, it was intended to prove vaccine virus in the peripheral vein blood.

However, if the blood of peripheral veins shows as much or at least nearly as much quantity of vaccine virus as the heart's blood, the conclusion that vaccine virus precipitates on the skinny tissues becomes, highly doubtful. It is sure that, in the case of heart puncturing the needle passes through the skin, thick muscular tissues, and, in rare cases, the lung-tissues. In such cases, if the vaccine virus is deposited in such tissues it may easily be carried out-side through the agency of the needle. Such argument gets more encouragement from Casagrandi (3), who holds the opinion that the lung-tissues have the strongest affinity to vaccine virus.

The consideration of these arguments increased the more the necessity of proving vaccine virus in the blood of peripheral veins. With these objects in view the following experiment was carried out:

Table VI.

Rabbit		Date of injection		Injected material	Vaccinal changes of testicles after the inoculation		Termination	Date of revaccination and its result.	Result of inoculation on skin with resp testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves.
96	1830	Jan. 23.	2	ear-vein blood	+	(—)	Jan. 26. †			
97	1815	Jan. 24.	3	do	+	(—)	living	Feb. 11. skin —	+	+
100	1800	Jan. 25.	4	do	+	(—)	living	Feb. 11. skin —	+	+
104	1500	Jan. 26.	5	heart blood	+	(—)	living	Feb. 11. skin —	+	+

106	1370	Jan. 27.	6	heart blood	+	(−)	living	Feb. 11. skin −	+	+
111	1950	Jan. 28.	7	ear-vein blood	+	(−)	living	Feb. 11. skin −	+	+
112	1920	Jan. 29.	8	do	+	(−)	living	Feb. 11. skin −	+	+
117	1560	Jan. 30.	9	do	×		Feb. 5. †			
120	1690	Jan. 31.	10	do	+	(−)	living	Feb. 20. skin −	+	+
123	2200	Feb. 1.	11	heart blood	±	(−)	living	Feb. 20. skin −		
129	−	Feb. 2.	12	do	−	(−)	living	March 18. skin +		

Two cubic centimeter of blood was obtained from the heart or ear veins of rabbit No. 91 mixed with a 4% citric solution in the proportion of one cubic centimeter of the blood to one tenth cubic centimetre of the fluid. One cubic centimeter of the mixture was injected into each of the testicles at a time.

Experiments described above conclusively demonstrate that vaccine virus obviously existed in the blood obtained from the heart and ear-veins of the rabbit No. 91, during the period covering from the third to the eleventh day after vaccination. Two rabbits (No. 96 and 117) which died early in the half way of experiment must be excepted from the consideration.

The above table which summarizes the results of the experiment is conclusive in demonstrating that, in the first place, the vaccine virus grown on the calf skin can also enter into the blood circulation as well as, that which cultivated in the rabbit testicle; and in the second place, no difference can be found in the results between the blood in the heart and that in the peripheral veins. It is an opinion generally accepted today that even the vaccine virus (cow-pox virus) discovered naturally is nothing but the variola virus which had been transferred to calf skin through accidental infection and propagated on it. Consequently the repetition of experiments by employing natural vaccine (natural cow-pox virus) instead of variola vaccine is not necessary. However, the variola vaccine which I employed is rather young in its generation. This fact compels me to encounter the attack that comes from the prevailing opinion that, the variola vaccine, of younger generation, or so-called "Jung Variola vaccine" is exceedingly stronger in its virulence (as for example, in the human body, it produces severe areola, and marked swellings on the regional lymphatic glands, temperature rises high.) and its immunity continues longer. Thus it is not unnecessary to investigate the influence of the number of generation of the virus to the invasion of vaccine virus into the blood-circulation.

Hence, in the following experiment the vaccine lymph issued by the Kitazato Institute was employed. For, the vaccine issued by the Institute was imported from abroad a long time ago, and, so far as I have been told, since then it has been passaged exclusively on the calf skin.

Besides the objects mentioned above, the ascertainment of the respective quantity of the virus contained in the heart-blood and earvein-blood was also attempted in the experiment. For this purpose, the left testicle of each rabbit was injected with the heart-blood and the right one with the earvein-blood.

Experiment V.

Rabbit No. 58 (weight 1700 grs.)

Dec. 23, 1919. One insertion was made on the skin of the nasolabial part with the vaccine virus issued by the Kitazato Institute. Dec. 25, produced papule of a reddish color. Dec. 27, the head of the papule changed into pustule. Dec. 29, marked swelling was observed at the site of vaccination. On the same day, during the operation of puncture, the rabbit died from internal hemorrhage. From the following day of vaccination to the day of death (the sixth day), blood was tested every twenty-four hours. The results of the injection with the blood so obtained are summarized in the following table:

Table VII.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of testicles after the inoculation		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbit	Calves
62	2100	Nov. 24.	1	1 ccm. of blood obtained by heart or earvein puncture, was made into a 0.4% citrate blood, 1 ccm of which was injected into each of the testicles (in left testicle heart blood, in right ear-vein blood.)	R — L —		living	Jan. 23. skin —		
63	1600	Nov. 25.	2		R — L +	(—)	living	Jan. 17. skin —	L +	L +
64	1700	Nov. 26.	3		R + L +	(—) (—)	Jan. 9. †		R + L +	R + L +
65	2050	Nov. 27.	4		R + L +	(—) (—)	living	Jan. 17. skin —	R + L +	R + L +
66	2200	Nov. 28.	5		R + L +	(—) (—)	Jan. 9. †		R + L +	R + L +
67	1800	Nov. 29.	6		R + L +	(—) (—)	Jan. 10. †		R + L +	R + L +

The heart and peripheral vein blood which was obtained on the next day

of vacccination shows no special signs on the inoculated testicles. But the blood taken aut from the second to the sixth day after inoculation all presented marked change on the testicles in the typical manner except one case which had been injected with the ear blood obtained on the second day after the vaccination. In other words, both the heart-blood and earvein-blood have no difference in their containing of vaccine virus.

In short, the results from the preceding five experiments conclusively demonstrate that, *firstly*, vaccine virus is obviously detectable, for a certain period of time, in the blood obtained from the rabbits which had been vaccinated on the skin; *secondly*, the area of the inoculated site or the kind of vaccine virus makes no difference whatever in the results; aud *finally*, the quantity of vaccine virus contained in blood is identical in both the heart and the earvein-blood.

Chapter IV.

Relation of Pustule-formation to Invasion of Vaccine Virus into the Blood-Circulation.

In the preceding chapters the invasion of vaccine virus into the blood-stream has been conclusively ascertained. In this chapter, however, the results of the further experiments are to be described. In order to ascertain the relation of the pustuleformation to the invasion of vaccine virus into the blood-stream, the following experiments were carried out. The outside of the eartip of the rabbit was shaved. and inoculated with variola vaccine. Then the ear so inoculated was out off from the root either before or after the formation of pustules. Next, blood was obtained from the rabbit, and tested minutely by injecting it into the testicles of other rabbits.

Experiment VI.

Rabbit No. 87 (weight 1350 grs.)

Jan. 20, 1920. Both eartips were shaved, and only the left one was inoculated by means of the Calmette and Guérin method with the variola vaccine (calf) in the fifteenth generation. Jan. 21, no change was observed on both eartips. The inoculated site of the ear was cut off from its root. Feb. 20, the nasolabial part and the each testicle were reinoculated with one-half cubic centimetre of the same vaccine, which showed no vaccinal effect. Blood was obtained every day during the period from the next day of the inoculation to the end of the thirteenth day, to be tested whether it contained vaccine virus. The following table gives the summarized results of the experiment:

Table VIII.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of testicles after the inoculation		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves.
89	1500	Jan. 21.	1	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood 1 ccm. of which was injected into each of the testicles.	−		Jan. 29. †			
92	1400	Jan. 22.	2		−		living	Feb. 11. skin +		
94	1700	Jan. 23.	3		−		living	Feb. 11. skin +		
98	1850	Jan. 24.	4		+		living	Feb. 11. skin −		
102	2000	Jan. 25.	5		+	(−)	living	Feb. 11. skin −	+	+
105	1200	Jan. 26.	6		+	(−)	living	Feb. 11. skin −	+	+
108	1580	Jan. 27.	7		+	(−)	living	Feb. 11. skin −	+	+
110	1740	Jan. 28.	8		+	(−)	living	Feb. 11. skin −	+	+
114	1700	Jan. 29.	9		×		Jan. 31. †			
116	1640	Jan. 30.	10		±		living	Feb. 20. skin −		
119	1700	Jan. 31.	11		−		living	Feb. 20. skin +		
121	1980	Feb. 1.	12		−		living	Feb. 20. skin +		
127		Feb. 2.	13		−		living	Feb. 20. skin +		

Rabbit No. 88 (weight 1500 grs.)

Jan. 20, 1920. The left eartip was inoculated by Calmette and Guérin method with the variola vaccine (calf) is the fifteenth generation. On the following day, no change was detected on both the inoculated and controling sites. Jan. 22, disseminated crimson spots were observed here and there on the inoculated site, which, on being held to the light, presented marked congestion and dark reddish knots, Jan. 23, the crimson spots turned into fairly raised small papule-like knots. On the same day, the left ear was cut off. Feb. 20, the nasolabial part and the both testicles (0.5 ccm.) were reinoculated. The reinoculation showed

no vaccinal changes, blood was obtained as usual during the days beginning from the next day of vaccination and ending on the fifteenth day, and was tested to determine the existence of vaccine virus in it. In the following table the results are well summarized.

Table IX.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of Inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
90	1800	Jan. 21.	1	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm of which was injected into each of the testicles	−		living	Feb. 11. skin +		
93	1750	Jan. 22.	2		−		living	Feb. 20. skin −		
95	1750	Jan. 23.	3		−		living	Feb. 11. skin +	−	−
99	1480	Jan. 24.	4		+	(−)	living	Feb. 11. skin −	+	+
101	1980	Jan. 25.	5		±∴	(+)	living	Feb. 11. skin +	−	−
103	1240	Jan. 26.	6		+	(−)	Feb. 5. †		+	+
107	1300	Jan. 27.	7		+	(−)	living	Feb. 11. skin −	+	+
109	1560	Jan. 28.	8		+	(−)	Feb. 2. †		+	+
113	1600	Jan. 29.	9		+	(−)	living	Feb. 20. skin −	+	+
115	1960	Jan. 30.	10		+	(−)	living	Feb. 20. skin −	+	+
118	1650	Jan. 31.	11		+	(−)	living	Feb. 20. skin −	+	+
122	2100	Feb. 1.	12		+	(−)	living	Feb. 20. skin −	+	+
128	1400	Feb. 2.	13		±		Feb. 13. †			
130	1550	Feb. 3.	14		−		living	Feb. 20. skin −		
134	1950	Feb. 4.	16		−		living	Feb. 20. skin +		

Note ±∴ indicates the [illegible] in the [illegible] testicle.

Rabbit No. 125 (weight 1750 grs.)

Feb. 2, 1920. The left eartip was inoculated as above, with the variola vaccine (calf) in the fifteenth generation. Feb. 6, confluent papule was observed on the inoculated site. Feb. 7, the ear was cut off from the root. Feb. 11, the rabbit died from some unknown cause. The anatomical results presented no special change in the internal organs. By injecting the blood obtained from the rabbit before and after the time of the cutting-off, the containing of vaccine virus was investigated as shown in the following table:

Table X.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its method	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
136	1680	Feb. 6.	4	2 ccm. blood obtained by heart puncture, was injected material made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles.	+	(−)	Feb. 16. †		+	+
139	1550	Feb. 7.	5		+	(−)	living	March 3. skin —	+	+
143	2000	Feb. 8.	6		+	(−)	living	March 3. skin —	+	+
147	1750	Feb. 9.	7		±		living	March 3. skin —		
151	1700	Feb. 10.	8		−		living	March 3. skin +		

Rabbit No. 126 (weight 850 grs.)

Feb. 2, 1920. The left eartip was inoculated by the same method as in the preceding experiments, with the variola vaccine (calf) in the fifteenth generation. Feb. 9, typical confluent papule was observed at the inoculated site. Feb. 7, the cutting-off of the ear was done. Feb. 20, the reinoculation in the nasolabial part and in testicles (0.5 ccm.) was made, but it presented no vaccinal effect. Blood was obtained from the rabbit both before and after the cutting-off of the ear, and was tested by injecting it into the rabbit testicle. Following table gives the results of the experiment:

As demonstrated in the preceding tables, the vaccine virus in the blood obtained from the rabbit No. 87 on the day before the cutting-off of the ear and on the ninth day after inoculation, is unknown, because of the early death of the rabbits injected with it. But the blood, obtained from the same rabbit on the third, fourth, fifth, sixth, seventh and ninth day after its ear was cut off,

Table XI.

Rabbit		Date of Injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
137	1300	Feb. 6.	4 dys.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles.	±		living	March 3. skin + rud		
140	1250	Feb. 7.	5 dys.		+		Feb. 15. †		+	+
144	2180	Feb. 8.	6 dys.		+	(−)	living	March 3. skin −	+	+
146	1450	Feb. 9.	7 dys.		+	(−)	Feb. 23. †		+	+
152	1700	Feb. 10.	8 dys.		−		living	March 3. skin +		
154	2100	Feb. 11.	9 dys.		−		living	March 3. skin +		

presents typical vaccinal changes when injected into other rabbits testicles, with a few exceptions; the exceptions being the bloods of the first, second tenth, eleventh and twelfth day, after the ear was cut off, which show no reaction in the rabbits' testicles injected with it. Rabbit No. 88 (Table IX) presents the virus in the blood obtained from it on the second day after inoculation. But that of third day presents no vaccine virus. In the blood of the fourth day (the first day after the ear was cut off) demonstrates vaccine virus, but that of the following fifth day (the second day after the ear was cut off) is again not demonstrable. With the exception of the above stated cases and that of the last day of experiment, all the rabbits' testicles injected with the blood obtained from the same rabbit on each of the days from the sixth to the fourteenth day, proves vaccine virus, providing only the rabbit which was injected with the blood of the thirteenth day is unknown, because of the early death of the rabbit injected with it.

Rabbit No. 125 (Table X) demonstrates vaccine virus during the days from the fourth day (the day before the cutting-off of the ear) to the seventh day after the vaccination.

Rabbit No. 126 (Table XI) plainly presents vaccine virus in its blood of each of the days from the fifth (the days before the cutting-off of the inoculated ear) to the seventh day (Two days after the cutting-off). Even the blood of the fourth day (two days before the cutting-off) shows on the rabbit testicles some vaccinal changes tough slight, and the revaccination reacted with imperfect pustulation.

Thus the results of the above experiments demonstrate the following facts: Vaccine virus appears and propagates day by day in the rabbit's body, even though the inoculated ear is cut off before the formation of pustules and before vaccine virus are detectable in its blood. And, on the other hand, even if the inoculated ear is cut off after the formation of pustules, vaccine virus is detectable in the blood in no less quantity than before the cutting-off of the inoculated ear.

These materials lead us up to the conclusion, that the inoculated vaccine virus invades into the blood before pustules are formed on the inoculated site. Thus invasion of vaccine virus takes place, before the cutting-off of the ear, and at the same time the virus begins its propagation somewhere in the vaccinated organism besides on the vaccinated skin site. If not so, it becomes incomprehensible that the vaccine virus is found two-days after the cutting-off of the inoculated site (rabbit No. 87).

The negative effects of the revaccination which rabbit No. 87 and 88 presented, when their previous vaccinated parts were cut off before the formation of pustules, agree with what Kraus and Volk[16] obtained in their experiments. Kraus and Volk inoculated monkeys with vaccine virus, then cut off the inoculated skin site before any vaccinal changes occurred. They found that the monkeys had become perfectly immune. However, the results which Kraus and Volk obtained from their experiments are greatly liable to various doubts. For, Nobl[19] supposed that, in case of subcuteneous vaccination, vaccine virus may possibly multiply in the subcutaneous collagenic tissues, on the ground that, Hückel[12] had discovered Guarnieri's bodies in the connective tissues of Cornea. Moreover, Knöpfermacher[17] and v. Provazek maintains that the infiltration arising from subcutaneous vaccination has at least something to do with immunity.

In my experiments, however, these doubts can never arise, because the vaccinated ear is cut off from the root which is much apart from the site of vaccination. With all these precautions for avoiding probable doubts, immunity has been brought about in typical manners.

Now I am in a position to conclude that vaccine virus invades the blood circulation before marked changes are observed at the vaccinated sites, so that the vaccinated organism becomes immune.

Chapter V.

Fate of Vaccine Virus Injected into the Vein.

Calmette and Guérin[2] insisted at first that the vaccine virus injected into the vein perishes in the moment it enters into the blood or internal organs, but in the skinny tissues it continues its life during twenty-four hours after intravenous inoculation. After some minute experiments, v. Prowazek and Yamamoto[29] have worked it up to an assertion which is widely accepted at present. This fact is the only basis upon which the investigators against the assertion of invasion of

vaccine virus into the blood-circulation are standing.

If, however, the assertion described in the last paragraph is proved to be true, the results of my experiments (Experiment I, II, III, IV and V) may be understood as showing that vaccine virus invades the blood-circulation incessantly from the pustules of the vaccinated skin site, and, as Hallwachs[11] maintain, it precipitates in the skin covering all over the body. However, such interpretations may not solve the question arising from Experiment VI, in which vaccine virus is proved to be still existing in the blood, even after the vaccinated site was cut off.

In order to solve the above question and to assert, that the virus must propagate in the body besides the vaccinated skin site, the following experiment was carried out:

Experiment VII.

Rabbit No. 131 (weight 1780 grs.)

Variola vaccine employed for the purpose was rabbit's testicular culture of B. strain in the twenty-sixth generation; the virulence of this vaccine being a little weaker than that of the thirty-third generation (strain Kawasaki).

(On the rabbits' skin-surface, a 1 : 5000 dilution of this virus produces partly confluented and partly disseminated papules. In the testicles, 0.5 cubic centimeter of a 1 : 100,000 dilution shows vaccinal changes after one week incubation.)

Feb. 3, 1920. The ear vein was inoculated with one cubic centimeter of a 1 : 50 dilution of the above mentioned vaccine. In order to avoid the propagation of the vaccine virus on inoculated skinny tissues site, the ear was cut off from the root immediately after the inoculation was finished. Feb. 10, both testicles showed swelling and marked edema was observed on the scrotum. The testicular parenchyma was slightly swollen and indurated. Feb, 14, the scrotal edema was nearly gone, leaving behind only vague traces, while the both testicular parenchyma still had hard, small knots. Feb. 15, both testicles were castrated.

Anatomical changes of castrated testicles.

The scrotum was only very slightly edematous, and tunica vaginalis was slightly congested. A few yellowish granula of about the size of millet or rice were observed in the parencyma of both testicles, which showed no marked congestion, nor were they hard and brittle. Bacteriological tests of the testicles were all negative. The emulsion prepared from this rabbit's testicles was inoculated on the rabbits' and calves' skin-surface which produced pustules.

March 3, on the skin of nasolabial part was made a reinoculation and presented negative effects. Blood was obtained during the days from the next

day of vaccination till the thirteenth day. By the method of testicular injection, the containing of virus in the blood was minutely tested. The following table shows the summarized results of the experiment.

Table XII.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
132	1340	Feb. 5.	1 dys.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles.	+	(−)	Feb. 11. †		+	+
133	1840	Feb. 5.	2 dys.		+	(−)	living	March 3. skin —	+	+
135	1300	Feb. 6.	3 dys.		+	(−)	living	March 3. skin —	+	+
138	1400	Feb. 7.	4 dys.		+	(−)	living	March 3. skin —	+	+
142	1750	Feb. 8.	5 dys.		+	(−)	Feb. 15. †		+	+
145	1670	Feb. 9.	6 dys.		+	(−)	March 2. †		+	+
153	1450	Feb. 10.	7 dys.		+	(−)	Feb. 24. †		+	+
155	1860	Feb. 11.	8 dys.		+	(−)	Feb. 18. †		+	+
156	1700	Feb. 12.	9 dys.		−		living	March 3. skin —		
160	1450	Feb. 13.	10 dys.		+		Feb. 29. †		+	+
164	2230	Feb. 14.	11 dys.		−		living	March 3. skin —		
166	2350	Feb. 15.	12 dys.		−		living	March 3. skin —		
169	1300	Feb. 16.	13 dys.		−		living	March 3. skin —		

Rabbit No. 174 (weight 1500 grs.)

Feb. 18, 1920. The ear vein was inoculated with one cubic centimeter of a 1 : 200 dilution of the vaccine lymph issued by the Kitasato Institute. The preparation of the material for the experiment was done by mixing the virus

with sterile physiological salt solution in the proportion of one capillar quantity (a quantity available for ten persons) of the former to four cubic centimeter of the latter.

Immediately after the inoculation the ear was cut off from the root. No change had been detected in the testicle, skin and conjunctiva until Feb. 22, on which day the rabbit was slaughtered. The anatomical investigations showed no marked changes in the internal organs some part of which was taken out at the same time, kept in the refrigerator for one day, and on Feb. 23, the organs were made into an emulsion with sterile physiological salt solution in the proportion one gram of the tissue to four cubic centimeter of the fluid. The organ emulsion so prepared was injected into the testicles of rabbits at the ratio of one cubic centimeter for each testicle. The containing of vaccine virus was carefully tested in the blood obtained every day from the day of inoculation to the day of slaughter. The results are shown in the following table:

Table XIII.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and ist result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
179	1550	Feb. 19.	1 dy.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles.	+	(−)	living	March 3. skin —	+	+
184	1730	Feb. 20.	2 dys.		+	(−)	March 3. †		+	+
185	2050	Feb. 21.	3 dys.		+	(−)	March 1. †		+	+
188	2250	Feb. 23.	4 dys.	mesenteric glands	+	(−)	March 2. †		+	+
189	1220	Feb. 23.	4 dys.	spleen	+	(−)	living	March 15. skin —	+	+
190	1280	Feb. 23.	4 dys.	lung	+	(−)	living	March 15. skin —	+	+
191	1630	Feb. 23.	4 dys.	liver	+	(−)	living	March 15. skin —	+	+
192	1930	Feb. 23.	4 dys.	bone-marrow	+	(−)	living	March 15. skin —	+	+
193	1550	Feb. 23.	4 dys.	testicle	+	(−)	March 1. †		+	+
194	1400	Feb. 23.	4 dys.	kidney	+	(−)	March 4. †		+	+
195		Feb. 23.	4 dys.	heartblood	+	(−)	March 3. †		+	+

Rabbit No. 175 (weight 2010 grs.)

Feb. 19, 1920. The ear vein was injected with 1 ccm. of 1 : 1,000 dilution of the usual variola vaccine virus (calf) in the fifteenth generation. (One capillar quantity available for five porsons-of the virus had been diluted in ten cubic centimeter of sterile physiological salt solution). Immediately after the inoculation the ear was cut off from the root. Until Feb. 28, no change was detected in the testicle, skin and conjunctiva. On the same day, the rabbit was slaughtered, and dissected. No marked change was observed in the internal organs. The blood obtained from the rabbit from two hours after inoculation to the sixth day, was minutely tested in the usual way. The following table is the summarized results of the experiment:

Table XIV.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
180	2300	Feb. 20.	2 hs.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, of which was injected into each of the testicles.	×		Feb. 22. †		−	−
183	1700	Feb. 20.	1 dy.		±		living	March 3. skin + rud		
187	1300	Feb. 21.	2 dys.		+	(−)	living	March 18. skin −	+	+
197	2130	Feb. 23.	4 dys.		+	(−)	March 3. †		+	+
199	2130	Feb. 24.	5 dys.		+		living	March 18. skin −		
200	2050	Feb. 25.	6 dys.		+	(−)	living	March 18. skin −	+	+

Rabbit No. 176. (weight 1820 grs.)

Feb. 19, 1920. The ear vein was injected with one culbic centimeter of a 1 ; 2,000 dilution of the usual variola vaccine (calf) in the fifteenth generation (The dilution was prepared by mixing one capillar quantity available for five persons of the virus with twenty cubic centimeter of sterile physiological salt solution). The ear was cut off in the usual manner. The rabbit was slaughtered on Feb. 27, until that day no change was detectable in the testicle, skin and conjunctiva. On dissection no change was observed in the internal organs, excepting somd knots in the spleen and liver. The mesenteric and axillary glands,

showed no swelling but was a little gluey. These organs were kept in the refrigerator. March 1, some part from each organs were made into an emulsion with sterile physiological salt solution in the proportion of one gram of the tissue to four cubic centimeter of the fluid which was injected into other rabbit testicles at the ratio of one cubic centimeter for each testicle. Blood was obtained from two hours after intravenous inoculation till the eighth day on which the rabbit was slaughtered. The containing of vaccine virus in the blood and organs was tested by the testicular cultivation, as demonstrated in the following table.

Table XV.

Rabbit		Date of injection		Injected material and its method	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
Number	Weight				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
181	2050	Feb. 20.	2 hs.	2 ccm. of blood obtained by heart puncture, was made into a 0.4% citrate blood, 1 ccm. of which was injected into each of the testicles.	×		Feb. 23. †			
182	1180	Feb. 20.	1 dy.		−		living	March 3. skin −		
186	1730	Feb. 21.	2 dys.		+	(−)	March 3. †		+	+
197	2270	Feb. 23.	4 dys.		+	(−)	living	March 15. skin −	+	+
198	2100	Feb. 24.	5 dys.		+	(−)	Feb. 26. †		+	+
201	2030	Feb. 25.	6 dys.		+	(−)	living	March 15. skin −	+	+
207	1350	Feb. 27.	8 dys.		+	(−)	living	March 15. skin −	+	+
209	1730	March 1.	8 dys.	liver	+	(−)	March 9. †		+	+
210	1200	March 1.	8 dys.	spleen	+	(−)	living	March 15. skin −	+	+
211	2000	March 1.	8 dys.	bone-marrow	+	(−)	living	March 18. skin −	+	+
212	1800	March 1.	8 dys.	mesenteric gland	+	(−)	living	March 18. skin −	+	+
213	1430	March 1.	8 dys.	testicle	+	(−)	living	March 18. skin −	+	+
214	1250	March 1.	8 dys.	kidney	+	(−)	March 9. †		+	+

215	1920	March 1.	8 dys.	lung	+	(−)	March 5. †		+	+
216	1430	March 1.	8 dys.	brain and cord	+	(−)	March 4. †		+	+
217	1540	March 1.	8 dys.	muscle	+	(+)	living	March 18. skin −	+	+

In the above Experiment VII, rabbit No. 131 (Table XII) was inoculated with a fairly big quantity of vaccine virus. In this case, its blood obtained on the next day of inoculation was contained the virus, but presented very moderate changes on the testicles, and the incubation was also long. But the blood during the days from the second day to the tenth day contained the virus and showed strong changes in the testicles and shorter incubation.

Rabbit No. 174. (Table XIII) was also inoculated intravenously with a comparatively large quantity of vaccine virus. Vaccine virus was contained in the blood of the days from the next day of inoculation to the fourth day, and the lung, liver, kidney, bone-marrow, testicles and mesenteric glands taken out on the last day, presented the vaccinal changes on being injected into the testicles. Considered only from the point of view of incubation and testicular changes, the quantity of vaccine virus in the internal organs may be said to be larger than that in the blood.

Rabbit No. 175. (Table XIV) and No. 176 (Table XV) were inoculated with small quantity of vaccine virus. These rabbits showed no vaccine virus in the blood obtained on the following day of inoculation; and vaccine virus in the blood of two hours after inoculation, was unknown, because of the early death of the rabbit which was injected with it. With the exception of these instances, the blood of rabbit No. 175 and No. 176 contained vaccine virus in all other cases. In the organs, e. g., spleen, liver, bone-marrow, etc., obtained from No. 176 also showed vaccine virus.

Generally speaking, the testicles injected with the blood obtained from both rabbit No. 175 and 176 on the second and third day after inoculation showed comparatively weaker reaction and longer period of incubation; while those injected with the later blood reacted very strongly and with short periods of incubation. Again, the organ emulsion was generally stronger in its virulence than the blood obtained on the same day; especially, it is interesting to note that, the organ emulsion prepared from rabbit No. 174 (four days after inoculation), showed longer period of incubation than that prepared from rabbit No. 176 (eight days after inoculation).

The conclusion which the preceding experiments lead us to is quite the reverse of the conclusion which Calmette and Giérin,[28] and specially, v. Prowazek and Yamamoto[29] have obtained. The latter inculated into the ear-vein of rabbits with two cubic centiweter of a 1 : 30 dilution of vacccine lymph. (viz., a 1 : 180

dilution of stock lymph). After the lapse of a certain period, they inoculated on the cornea of other rabbits with the blood or organ emulsion obtained from the rabbit above mentioned. No vaccinal reaction was detected on the cornea, excepting only in those rabbits which had been inoculated with the blood or organ emulsion obtained from the rabbit early in the first few hours after inoculation. The former, on the other hand, inoculated a large quantity of vaccine virus into the vein of rabbits, and then, inoculated on the skin-surface of other rabbits with the blood and organ emulsion obtained from those rabbits during the days from the first to the fifth day after inoculation. But, they found all results negative.

Whilst, in my experiments, as shown in the previous paragraphs, in the blood of the first few days are detectable either no vaccine virus (when the inoculated quantity of virus is small) or very small quantity of virus (when the inoculated quantity is large). However, as time goes on, vaccine virus appears and propagates more and more, and rabbit No. 131, for example, demonstrates vaccine virus in its blood until ten days after inoculation. Moreover, the organ emulsion of the eighth day is stronger in its vaccinal activity than that of the fourth day after the intravenous inoculation. Though it is regrettable, owing to the death of the rabbits inoculated with the blood obtained from rabbit No. 175 and 176 at two hours after inoculation, it is impossible to determine whether vaccine virus in the blood once disappeared and comes out again, or it has existed in a very small quantity from the first.

Anyhow, there is no doubt that the vaccine virus begins propagation somewhere in the body during a certain period of time after inoculation. Where does it propagate is still an unsolved question. But it may well be imagined that the propagation takes place in the internal organs, and not or very little in the blood, because of the fact that the quantity of the virus in the internal organs is greater than that of the blood of the same day.

Moreover, the arguments of Calmette and Guérin, and especially, v. Prowazek and Yamamoto are highly defective in the following few points.

Firstly, in their experiments, the former employed the rabbit skin, while the latter used the cornea for the site of inoculation. They considered no doubt that the skin or cornea was the most sensitive to vaccine virus. But I maintain, as written at the start, that the skin and cornea the most unsuitable for testifying very small quantity of vaccine virus, for only the least quantity is capable of touching the injured spot on the covering cell, to say nothing of the impossibility of preventing accidental infection of the virus.

Secondly, They conclude that the inoculated vaccine virus in the vein perishes the moment it enters into the internal organs, while in the skinny tissues it can continue life for a certain period of time, and then exterminate there sooner or later. This conclusion is entirely based upon the results of their experiments, in which the covering skin presented pustules on the injured spots only during twenty-four hours (Calmette and Guérin) or forty-eight hours (v. Prowazek and

Yamamoto) after intravenous inoculation, no formation of pustules could be detectable after that. But this conclusion can not be true, since, as I pointed out in the last paragprah, great defects are underlying in the method of experiments. Therefore, the pustules formation on the epithelium should not be attributed to the vaccine virus precipitating in the skinny tissues, because it may be highly probable that vaccine virus cirulating with the blood may form pustules on the skin. Moreover, the fact that the pustules could not be produced after twenty-four or forty-eight hours, may be due to the imperfect immunity which had been brought about. This fact can also be clearly detectable in the case of successive vaccination

In reference to the above investigations, the following statements may be added, though it has nothing to do with my present task. It is worthy of nothing that the vaccine virus which was inoculated into the vein of rabbit No. 131 of experiment VIII caused testicular swelling, while in other cases no change was detectable. Rabbit No. 131 was of course inoculated with a bigger quantity of vaccine virus than in other three cases, aud this was undoubtedly an important cause for testicular swelling. But the difference in the nature of vaccine virus should not be lost sight of. The vaccine virus inoculated into rabbit No. 131 had for a long time been cultivated in the rabbit testicle.

According to the results of Kii's experiments (not yet published) that kind of vaccine virus nearly always causes testicular swelling when injected into the rabbit veins, while the usual vaccine never causes such effects. Consequently, it may be reasonable to conclude that the vaccine virus which I employed in my experiment was also one which had been adopted to the rabbit testicle.

Chapter VI.

On Question whether Vaccine Virus has Affinity to Skinny Tissues

The preceding experiments are conclusive in showing the fact that the vaccine virus inoculated directly into the vein does not perish but propagates in the inoculated body. A question naturally follows whether or not the vaccine virus inoculated on the skinsurface can also be proved to be present in the internal organs as well as by intraveneous inoculation. This question may be answered in the affirmative by concluding from the results obtained in the preceding experimets. But exact and minute details can not be given except through a series of experiments specially elaborated for that purpose. In the preceding experiment I have not examined of vaccine virus containing in the skinny tissues. In the following experiments, therefore, the non-inoculated parts of the vaccinated rabbit skin were carefully examined so as to detect vaccine virus, the work being the reassurance of the conclusion obtained from Experiment IV and V shown in Chapter III.

Experiment VIII.

Rabbit No. 347. Sept. 10, 1920. One insertion on the skin of the nasolabial part was made by stab inoculation with variola vaccine virus of rabbit's testicular culture in the thirty-seventh generation. Sept. 14, characteristic pustule was detected. The animal was slaughtered on the same day after 2 ccm. of blood had been obtained from it. No change was observed in the internal organs. Each organ was made into a 1 : 5 salt solution of emulsion. The blood and emulsion which after being kept in the refrigerator for 3 or 6 days were injected into the rabbits' testicles. The results are shown in the following table : —

Table XVI.

Rabbit Number	Date of injection		Injected material in the testicles	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
408	Sept 17.	4 dys.	skin	±	(−)	living	Sept. 28. skin + rud	±	
409	Sept 20.	4 dys.	heartblood	−		living	Sept. 28. skin + rud		
410	Sept 17.	4 dys.	menenteric glernds	+	(−)	living	Sept. 28. skin −	+	
411	Sept 17.	4 dys.	kidney	+	(−)	living	Sept. 28. skin −	+	
412	Sept 17.	4 dys.	muscle	−	(−)	living	Sept. 28. skin +	−	
413	Sept 17.	4 dys.	tymus	−	(−)	living	Sept. 28. skin +	−	
414	Sept 17.	4 dys.	liver	+	(−)	living	Sept. 28. skin −	+	
415	Sept 17.	4 dys.	brain and cord	−	(−)	living	Sept. 28. skin +	−	
416	Sept 17.	4 dys.	bonemarrow	+	(+)	living	Sept. 28. skin −	+	
417	Sept 17.	4 dys.	lung	+	(+)	living	Sept. 28. skin −	+	
418	Sept 17.	4 dys.	spleen	+	(−)	living	Sept. 28. skin −	+	
419	Sept 17.	4 dys.	testicle	+	(−)	living	Sept. 28. skin −	+	

Note: Iccm of testmaterial was injected into each of the testicles.

Rabbit No. 420. Sept. 30, 1920. One insertion on the skin of the nasolabial part was made by stab inoculation with an usual vaccine virus (calf) in the sixteenth generation. Oct. 4, characteristic papule was observed. Oct. 7, papule changed into pustule. 2 ccm. of blood was taken out from the heart by means of puncture. The animal was slaughtered in the same day. Each organ was made into a 1 : 5 salt solution of emulsion, which was injected into the testicles of sound rabbits. The results are shown in the table below : —

Table XVII.

Rabbit Number	Date of injection		Injected material	Vaccinal changes of the testicles after the injection		Termination	Date of revaccination and its result	Result of inoculation on the skin with resp. testicular emulsion	
				Induration edema etc.	Contaminating bacteria			Rabbits	Calves
422	Oct. 7.	7 dys.	heartblood	±	(—)	living	Nov. 4. skin —	+		
423	Oct. 7.	7 dys.	testicle	+	(—)	Oct. 15. †		+		
424	Oct. 7.	7 dys.	liver	+	(—)	Oct. 10. †		+		
425	Oct. 7.	7 dys.	kidney	±	(—)	living	Nov. 4. skin —	—		
426	Oct. 7.	7 dys.	lung	+	(—)	living	Nov. 4. skin —	+		
427	Oct. 7.	7 dys.	spleen	+	(—)	living	Nov. 4. skin —	+		
428	Oct. 7.	7 dys.	bone-marrow	+	(—)	living	Nov. 4. skin —	+		
429	Oct. 7.	7 dys.	brain and cord	—		living	Nov. 4. skin —			
430	Oct. 7.	7 dys.	skin	±	(—)	living	Nov. 4. skin +	—		
431	Oct. 7.	7 dys.	muscle	—		living	Nov. 4. skin +			
432	Oct. 7.	7 dys.	mesentere glands	—	—	living	Nov. 4. skin +			

Note: 1 ccm of testmaterial was injected into each of the testicles.

As shown in the Table XVI of Experiment VIII, rabbit No. 347 has conclusively indicated vaccine virus in its mesenteric glands, kidney, bone-marrow, lungs, spleen and testicles. The testicles inoculated with the skin emulsion and blood showed no characteristic changes. But the imperfect pustulation caused by

the reinoculation of vaccine virus indicates that vaccine virus had been present in small quantity in them. The fact that the testicles injected with blood showed imperfect pustules while any change was hardly detectable, may be due to the perishing some quantity of the virus by action of vaccinecidin during the deposit of six days in the refrigerator. Muscles, brain, cord and thymus presented no vaccine virus.

Rabbit No. 420 (Table XVII) showed vaccine virus in its blood and organs with the exception of mesenteric glands, muscles, skin and kidney.

As may be seen in case of direct injection into veins, a bigger quantity of vaccine virus is proved in organs than in blood, while the degree of frequency of positive manifestations of vaccine virus in the organs is less than that observed in the case of injection into vein.

Such differences may be due to the fact that, in case of percutaneous vaccination it may well be imagined that a small quantity of vaccine virus invades into blood very gradually, while in case of intraveneous injection a big quantity is injected at a time. This statement, however, needs still further investigations before it is asserted decisively.

With regard to the vaccine virus in the skinny tissues, rabbit No. 375 proved vaccine virus, though in a very small quantity, while rabbit No. 420 showed no virus whatever. The results obtained from this experiment combined with those from Experiment IV and V make the affinity of vaccine virus to the skinny tissues highly doubtful. Consequently, the statements made by Hallwachs,[11] can never be believed hastily, to say nothing of the arguments held by v. Prowazek and Yamamoto.[29]

Chapter VII.

On Process of Formation of Vaccinal immunity.

No decisive conclusion can yet be laid down with regard to the process of formation of vaccinal immunity, since I have still a few more questions that await solving. However, the accepted theories at present on vaccinal immunity all prove to be based upon the conclusion that "Vaccine virus does not generalizes all over the body."

v. Prowazek, whose theory in this connection is widely accepted at present, insisted first that the vaccinal immunity is the histogenic. And Jochmann[13] gives the following three points:

Das geht aus drei Momenten hervor:

1. Ist der eigentliche Beginn der Immunität unabhängig von dem Auftreten dieser Antikörper,
2. besteht die Immunität in den meisten Fällen noch lange, nachdem die Antikörper längst verschwunden sind
3. Ist das Vorkommen der viruliciden Antikörper im Blute von Menschen,

Affe und Kaninchen äusserst variable und keineswegs gesetzmässig.

Thus he maintains that the phenomena of immunity arise only in the skinny tissues, and not in the blood.

Süpfle[37] says in his article entitled "Das Wesen des Impfschutzes im Lichte der neueren Forschungen": —

> Das Lokalisiertbleiben der kutan inserierten Vaccineerreger in dem Epithelbereich der Haftstelle ist für die Auffassung von dem Zustandekommen des Impfschutzes wichtig. Es macht den Gedanken sehr wahrscheinlich, dass die Immunität gegenüber einen Erreger, der so ausgesprochen an das Epithel angepasst ist, auch eine Leistung des Epithel ist.

Referring many other instances, Süpfle further discusses that immune bodies in the blood are not produced in the blood, but transferred through blood from the epithel, and that immunity is effected noly on the cover-epithelium itself, and therefore, the internal organs have nothing to do with it.

In reality, as many investigators report, the immunity of the skin has nothing to do with appearance of vaccinecidin, because the former is brought about before the latter appears. The immune bodies are not contained in the blood in a regular quantity, but change freely according as the difference of the vaccinated organism. But it is wrong, however, to conclude from the above statements that the immune bodies have entered into the blood from the skinny tissues. For, on the contrary, I have proved the existence of vaccine virus in the blood and organs, it seems to be natural to consider that other organs and tissues may as well become immune and become able to produce immune-bodies. Investigators who maintain that, if one inoculates vaccine virus in the cornea on one side he will find immunity effected only on this side, and no immunity will be observed the skin and other cornea on the other side. Again, in the contrary, they insist, even though the skin becomes immune, by the percutaneous vaccination the cornea receives no effect from it. This is the only ground upon which they maintain that the vaccinal immunity is the histogenic immunity which arises only at the site of vaccination; and, therefore, the other organs and the tissue-fluid have nothing to do with it.

Süpfle further maintains: —

> Wir Können bei der kornealen Vaccine in geradezu klassischer Weise verfolgen, wie hier, wo jede Mitwirkung der Blutflüssigkeit ausgeschllossen ist überhaupt gar kein andere Möglichkeit in Betracht kommt, als dass Immunität einer seits zellulären Ursprungs ist, andererseits auch infolge eine zellulären Eigenschaft fortdauert.
>
> Es ist wohl berechtigt, aus diesen Erfahrungen, zusammengehalten mit unseren übrigen Kenntnissen, den Schluss abzuleiten, dass auch die Epithelzellen der Haut- und Schleimhautdecke die Fähigkeit haben, auf den Reiz des vaccinalen Antigens hin von sich aus, ohne Mitwirkung innerer

Organe, eine parasitizide Immunität zu gewinnen. Erfolgt die Ansiedelung der Erreger nicht auf der Korner, sondern im Epithel der Haut oder Schleimhaut, so fällt natürlich die ernährungs-physiologische Isoliertheit der Kornea mit ihrer eigenartigen Wirkung, dass die Immunität die anatomischen Grenzen des Korneaepithels nicht überschreiten kann, weg; der Blut- und Säfteaustausch ist über die ganze Epithellage des Organismus ungehindert, und die Immunität ergreift die gesamte Haut- und Schleimhaut oberfläche.

On the contrary, my experiments elaborated and repeated up till now, have conclusively proved that the vaccine virus inoculated on the skin, invades into the blood, and propagates in some organs and tissues. What then will successfully solve the question of immunity in relation to the skin and cornea?

From nutritic physiological and anatomical point of view, the cornea is entirely independent from the other organs and tissues. In consequence, antigen (dead or alive) cannot easily be brought to the cornea in ordinary cases. But, if the massive vaccination is made on the skin, or, as shown in Kraus and Volk's[16] experiments, if vaccine virus is inoculated into conjunctiva, the cornea of which and the skin will become imperfectly or sometimes perfectly immune. For, in these cases, antigen is brought about in the cornea in a quantity which is enough to make it immune.

In short, what I want to emphasize here in this connection is that the immunity is effected not only in the skin, but it is an active immunity of the entire organism. In this respect, however, my argument is wholly different from that of Pirquet[31] *and Hallwachs,*[11] *although I admit that the proper form of immunity is histogenic and the humoral immunity is but an auxiliary form.*

Chapter VIII.

Difference between Variola Virus and Vaccine Virus.

I have pointed out in the preceding pages that on the skin-surface inoculated vaccine virus propagates in the internal organs after its invasion into the blood. I have now come to the point of solving the question that, if the virus exist or propagates in the skinny tissues and internal organs, why vaccinal changes are not detectable in those parts of the body.

With the view of explaining the difference between varola and vaccine virus Pirquet[31] maintains: —

Das vaccine virus regt die Antikörperbildung in ähnlicher Weise an wie variolöse, vermehrt sich aber nicht so schnell.

Supfle,[29] on the other hand, sought the cause of the difference between variola virus and vaccine virus, in the difference in the intensity of propagation of each virus.

He writes: —

> In der Regel wird nach meiner Ansicht frühzeitig genug die Immunkörperbildung qualitativ and quantitativ ausreichen, um eine generalisierung der lokal inserierten Vaccineerreger unmöglich zumachen.

But, according to my opinion, when variola virus turns into vaccine virus by the passages through the animal tissues, the nature of the virus somewhat changes, though its antigenic power remains unchanged. *The virus decreases in its intensity of propagation as it increases in its threshold of stimulation. The intensity of propagation decreases, it is true, but not so much as Süpfle maintains, i. e., not so much as the propagation of the virus becomes limited only to the inoculated site.*

As the result of a great increase of threshold of stimulation, vaccine virus becomes in capable of pustulation without strong stimulation, while variola virus can pustules even with or without the least stimulation. In other words the threshold of stimulation of vaccine virus is higher than that of variola virus.

This hypothesis referred in the preceding paragraph will easily solve the question arising in relation to the Calmette and Guérin[2] and v. Prowazek and Yamamoto's[29] experiments, that the vaccine virus inoculated directly in the rabbit veins did not pustulate until the skin was given an awakening stimulation. And, at the same time, this hypothesis makes the question quite clear why, in my experiments, did not the vaccine virus produce any marked changes, in spite of its existence in the blood and organs.

Chapter IX.

Paragragh I.

Experiment on the Humanbody.

In my experiments rabbits were employed in all cases. How will it then be with human body? For the purpose of assertaining this, I inocultated my own child with vaccine lymph, and could prove vaccine virus in her blood at seventh days after the vaccination. The details will be published on the next opportunity, here it will be sufficient to add that the same results can be obtained from the experiment employing children.

Paragraph II.

Relation between Blood at Different Periods and Scrotal Edema.

As I pointed out in Chapter II, the terminal blood, on injection, presents more marked edema in the scrotum than in the blood of another period. What will be the cause for such a phenomenon? I am inclined to conclude that the

Table XVIII.—(A) Vaccine Virut in the Btood after Vaccination.

Kind of Vaccine virus	Inoculation: manner and site	Number of rabbit / after Vaccination	2 hrs.	8 hrs.	1 dy.	2 dys.	3 dys.	4 dys.	5 dys.	6 dys.	7 dys.	8 dys.	9 dys.	10 dys.	11 dys.	12 dys.	13 dys.	14 dys.	15 dys.	16 dys.	17 dys.	18 dys.	19 dys.
Variola Vaccine Rabbit testicular Culture	Percutaneous Inoculation on large area of backskin.	No. 1		−	+	+	+	+	+	†	+	+	+	+	+		+		+		−		−
		,, 14			†		+	+	+	+													
(23 rd Generation Kawasaki)	One insertion on rasbbed part	,, 15			−		+	+	+	+	+	+	+	−									
Vaccine lymph (Kitasato)	,,	,, 58			−	+	+	+	+	+													
Calf skin grown variola vaccine (15 th generation)	,,	,, 91				†	+	+	+	+	+	+	†	+	+	−							
,,	Inserted site was cut off before or after the formation of pustules	,, 87			†	−	−	+	+	+	+	+	†	+	−	−	−						
,,		,, 88			−	+	−	+	−	+	+	+	+	+	+	+	+	−					
,,		,, 125						+	+	+	+	−											
,,		,, 126						+	+	+	+	−	−										
Variola Vaccine Rabbit testicular culture (26 th generation ?)	Intravenous injection: 1 cc. in 1:50	,, 131			+	+	+	+	+	+	+	+	+	+	−	−	−						
Vaccine lymph (Kitasato)	1 cc. in 1:200	,, 174			+	+	+	+															
Calf skin grown variola vaccine (15 th generation)	1 cc. in 1:1.000	,, 175	†		+	+		+	+	+													
,,	1 cc. in 1:2.000	,, 176	†		−	+		+	+	+		+											

cause lies in the process of allergy which arises from vaccine virus and the antibodies in the blood.

Paragraph III.

Time of Appearance and Continuation of Vaccine Virus in the Blood of Vaccinated Rabbits.

This time and period shown in the subject may change according to the difference in the virulence of vaccine virus employed, and the animal used for the passage of virus from variola virus to vaccine virus. This, however, is devoted for explaining the individuality of the rabbit, the area of the inoculated site, and the mothod of inoculation, which are some of the condition which effect the time and period in question. The following table gives the difference in this respect.

Table XVIII.—(B)

Vaccine Virus in Organs after Vaccination.

Kind of Vaccine virus	Inoculation method and site	Organ / Number of rabbit	Skin	Muscle	Lung	Liver	Kidney	Spleen	Testicle	Mesenteric glands	Bonmarrow	Cord and brain	Thymus
Vaccine lymph (Kitasato)	4 dys. after intravenous inoculation	No. 174			+	+	+	+	+	+	+		
Calf skin grown variola vaccine (15th generation)	8 dys. after intravenous inoculation	,, 176		+	+	+	+	+	+	+	+	+	
Variola vaccine rabbit testicular Culture (37th G.K.)	4 dys. after one insertion on naso-labial part	,, 347	+	−	+	+	+	+	+	+	+	−	−
Calf skin grown variola vaccine (16th generation)	7 dys. after one insertion on naso-labial part	,, 420	−	−	+	+	−	+	+	−	+	+	

Note: + indicates the vaccine virus in testmaterial. − indicates the no vaccine virus in test material.

As shown in the above table, the virus is detectable in the blood from one day in the earliest to fifteen days in the latest after the vaccination.

Summary.

The testicles of normal rabbits, when injected with the blood obtatined from the rabbit, which had been the previously inoculated on the skin with vaccine virus, present the vaccinal changes in a typical manner. With very few exceptions, no bacteria was detectable in them; while the skin of rabbits and calves inoculated with the emulsion prepared from these testicles, showed marked vaccinal pustules. From these facts, it has also become conclusive that the

vaccine virus inoculated on the skin of rabbits regularly exists and propagates during a given time in the blood or internal organs.

Therefore, I have discovered in this connection, a very simple method of cultivating vaccine virus in pure state, free from all contaminating bacteria. In order to manufacture pure vaccine virus of testicular cultivation from vaccine lymph with bacteria, we have any longer no need to elaborate the complicated method introduced by Noguchi[21,22] or Paschen.[32] We have simply to inoculate the testicles of normal rabbits with the blood obtained from the vaccinated rabbit.

The method employed for my experiments entirely different from those which have been used up to the present time. The old methods are highly liable to accidental infections, because in them the inoculation of blood or organ emulsion is made an the skin or cornea, or provocating operation must be made on the skin or cornea of the vaccinated organism. But my intratesticular cultivation method, as explained above, may be injected the large quantity of test materials at a time and is perfectly free from accidental infection of vaccine virus, so that the results obtained from these experiments must entirely be authentic.

In conclusion it may necessarily be added that the theory of vaccinal immunity which have been accepted up till now should not be acknowledged hastily. The assertion, that vaccine virus is dermotrop, and vaccinal immunity is histogenic immunity brought about merely at the inoculated site, and that the immunity is limited only in the skinny tissues, and the antibodies is produced in the skinny tissues and carried into the blood-circulation; all such assertions have become doubtful. The vaccinal immunity must be at least the active histogenic immunity of the whole body, and not merely the immunity of the skin.

In closing, I express my heartiest thanks to Prof. Nagayo, Director, of our Government Institute for Infectious Diseases, the Tokyo Imperial University, and Prof. Hayashi, Previous Director of same Institute, for their kind instruction and encouragement. I am also very much indebted to Prof. Ishiwara and Prof. Kii for the kind help and guidance with which they favored me all through my work.

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Explanation of Figure.

Fig. 1. Edematous Hemorrhagic Form.
The left testicle shows marked vascular injection, edema and numerous hemorrhageous spots visible all over it.
The right testicle is a normal one (not inoculated with the test blood) shown for the controling purpose.

Fig. 2. The section of both testicles shown in Fig. I.

Fig. 3. Granular Form.
The testicle covered with two large and several small yellowish grey granula showing slight vascular injection on the surface. The section presents the same condition.

Fig 4. Mixed Form.
The testicle shows numerous yellowish grey granula and spotted here and there several small hemorrhagic area in the testicle. The section presents the same condition.

Fig. 5. The testicle of rabbit No. II.
On the 6 days after the testicular inoculation of test blood obtained from arabbit No. I. on the 8 days after the vaccination.
Both testicles shows marked swelling due to the induration and swelling of parenchyma and scrotal edema.

Fig. 6. The testicles of rabbit No. 23.
On the 9 days after the inoculation of test blood obtained from the rabbit No. 15 on the 4 days after the vaccination.
Both testicles show marked swelling, the left one indicating the fluctuation due to the severe skrotal edema.

Fig. 7. The castrated testicles of rabbit No. 281.
On the 7 days after the testicular inoculation of testblood obtained from my own child (about 3 months old) on the 7 days after the primary vaccination.
The right one shows marked edema and induration, slight vascular injection visible on the surface, left one presents no vaccinal changes.

Fig. 2.

Fig. 1.



Fig. 3.



Fig. 4.



(Dr. T. Ohtawara : Experimentaly Studies on the Process of Formation of Vaccinal Immunity.)

Fig. 5.

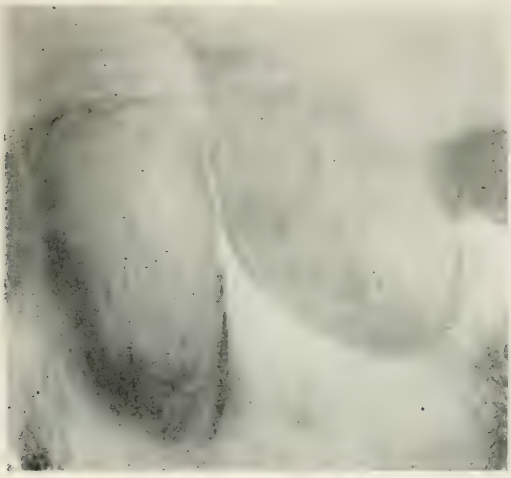


Fig. 6.

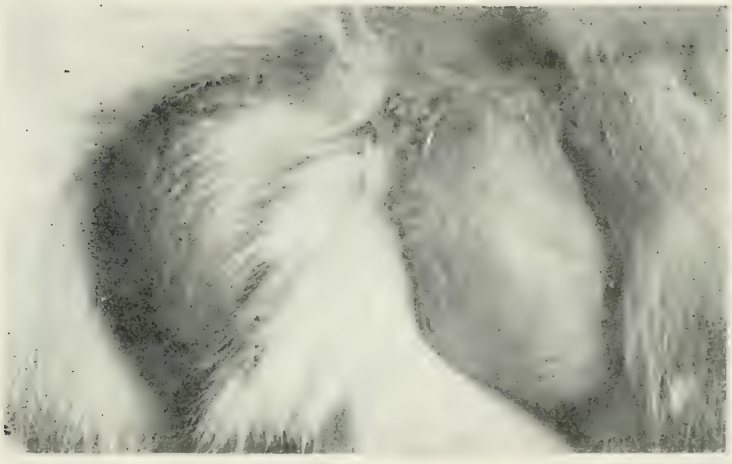


Fig. 7



(Dr. T. Ohtawara : Experimentaly Studies on the Process of Formation of Vaccinal Immunity.)

Ekzamenmetodo pri la efikeco de kupoksvakcino per enhaŭta inokulado ĉe kuniklo neperfekte imunigita.

De **Hisao Kasai.**

(El la laboratorio de la variola kaj kupoksvacinpreparada fako de la Instituto, la ĉefo D-ro Naoyoshi Kii.)

Malongigaĵo el la originalo antaŭe publikigita japane en "The Japanese Journal of Experimental Medicin," n-ro 2., voj. 6. Aprilo 1922.

Post precizaj eksperimentoj faritaj uzante multenombrajn bovidojn, Kii, en 1917, publikigis artikolon titolitan "Kontrolmetodo de kupoksvakcino per inokulado ĉe bovido imunigita neperfekte." En ĝi li asertis, ke la vakcino taŭga por inokulado ĉe homoj ne sole devas enhavi sufiĉan nombron da bovinvariolaj virusoj, sed ankaŭ ĉiu viruso devas posedi sufiĉan fortecon, kaj por juĝi, ĉu donita vakcino plenumas tian kondiĉon, estas bone, ke oni inokulu juĝotan vakcinon sur bovidon komenciĝantan imuniĝi kontraŭ bovinvariolo pro vakcinado farita antaŭ kelkaj tagoj, t. e. en neperfekte imunigita stato.

Mi antaŭe esploris, ĉu oni povus uzi kuniklon anstataŭ bovido en tiu ĉi kontrolmetodo, farante la inokuladojn imunigantajn kaj ekzamenantajn laŭ la metodo de Calmette kaj Guérin. Sed la ekzameno finiĝis tute malsukcesa, ĉar la reakcio elvokita per tia inokulado en neperfekte imunigita stado de la besto, estas tro malklara.

Depost 1916 Groth konsilas, ke juĝu vakcinon pri ĝia efikeco per enhaŭta inokulado ĉe kuniklo, ĉar tiu ĉi metodo alportas pli ekzaktan rezultaton, ol ordinara surhaŭta inokulado, kaj fine en 1921 li publikigis detalan informon.

Mi min okupis ekzameni pri kontrolmetodo de vakcino, enhaŭte inokulante al kuniklo imunigita neperfekte, unu metodo konsistanta en la kunigo de la pli bonaj punktoj de ambaŭ metodoj de Kii kaj Groth.

Antaŭeksperimentoj I.

Pri la loka reakcio elvokita de enhaŭte inokulita vakcino ĉe kuniklo, antaŭaj esplorintoj skribas diverse. Mi do faris antaŭ ĉio jenajn eksperimentojn, por lerni klinikajn kaj patologio-anatomiajn karakterojn de tiu ĉi reakcio, kaj eltrovi el la lokaj fenomenoj kelkajn konvenajn punktojn, sur kiuj apogante oni mezuras la fortecon de la reakcio.

La teknikoj kaj rezultatoj de la eksperimentoj:

1) 25 japanaj albinokunikloj pezantaj 2–3 kilogramojn estis uzataj.

2) 3 specimenoj de la kupoksvakcino, kies potenco estis ekzamenita per surhaŭtaj inokuladoj ĉe homoj kaj kunikloj, estis uzataj; la unua (vakcino A) la plej forta, la dua (v. B) mezgrada kaj la tria (v. C) malplej forta. Ĉiu vakcino estis diluata per fiziologia salsolvaĵo ĵus antaŭ la inokuladoj en proporcio de 1/1,000 kaj 1/10,000.

3) La inokulado estis farata ĉiam sur dorsa flanko. La inokulkampoj estis forrazataj kaj desinfektataj per alkoholo. La vakcinoj estis injektataj po 0.2 kubikcentimetro en la haŭtohiston per enŝprucigilo kun tre mallarĝa kanulo, aranĝante ĉiam en sama ordo, kun ĉirkaŭ 3 centimetroj da interspacoj reciprokaj. Deposte la irado estis ĉiutage observata precize.

4) La lokaj fenomenoj elvokitaj ĉe la injektita punkto entute akordiĝis kun tiuj skribitaj de Groth. La maldensiganta fluidaĵo de la vakcino injektita estis forsorbita post kelkaj horoj, kaj dum 24 horoj oni vidis nenian lokan reakcion. Poste sin montris specialaj ŝanĝoj en diversaj gradoj laŭ la propra forteco kaj la denseco de la vakcino injektita. En ĉiuj okazoj, komence aperis ruĝa makulo. Tiu ĉi punkto ŝvelis pli kaj pli, fine fariĝis la papilo, kiu iom post iom pli grandiĝis, ĝis ĝi atingis sian maksimuman grandecon post proksimume 24 × 4–5 horoj, kaj poste iom post iom ĝi malaperis. En la okazoj de la vakcinoj A kaj B, la papilo ne sole ruĝiĝis vigle kaj ĝia ĉirkaŭlimo estis klara, tiel ke oni povis mezuri ĝian diametron sen malfacilo, sed aukaŭ la haŭtoepitelio sur ĝia centro flavetiĝis pro variola degenero (ĉar tiu ĉi flaveta parto estas facile forskrapebla, kaj, se oni inokulas ĝin sur la haŭto de alia kuniklo, ĉiam aperas tipaj variolpustuloj, mi opinias, ke tiu ĉi haŭtoepitelio degenerinta estas komparebla kun tiu de la kunfluiĝantaj variolpustuloj naskitaj ĉe ordinala surhaŭta inokulado; pro tio mi malsupre ĉiam nomas ĝin "variolpustulo"). Plie, en pliparto de la okazoj, oni vidis rimarkindan hemoragion en tiea subhaŭta histo. Kontraŭe, en la okazo de la vakcino C, ĉiuj tiuj ĉi fenomenoj estis malpli fortaj, kaj oni ne povis ekzakte mezuri la larĝecon de la papilo pro ĝia malklara konturo.

Aliflanke, la sama vakcino reakciigis ne ĉiam samgrade ĉe kunikloj uzitaj. Tio ĉi dependas, kompreneble, de la individua diferenco en la sentemeco de kunikloj por bovinvariola viruso, kio estas tutkonata afero. Ĉe la enhaŭta inokulado, oni ja povas observi pli klare, kiel influas sur la reakcio tiu ĉi individua sentemeca diferenco de kuniklo, ol ĉe la surhaŭta inokulado. Eĉ inter nur 25 kunikloj inokulitaj de mi, mi vidis tian grandan malsamecon, ke troviĝas diversaj gradoj de reakcioforteco inter tiaj 2 ekstremoj, ke en iu okazo ĉiuj ĉi 3 vakcinoj aperigis nenian reakcion, dum en la alia ili ĉiuj tute vigle reakcigis preskaŭ samgrade. Laŭ nur ĉe malmultaj bestoj oni vidis ioman konkordon de reakcio.

5) Jen resumo el la eksperimentoj:

a) La papilo, la variola degenero de l'haŭtoepitelio sur la centro de la papilo kaj hemoragio en tiea subhaŭta histo estas la precipaj patologiaj ŝanĝoj vidataj kiel reakcio de la enhaŭta vakcinado ĉe kuniklo.

b) Ĉar, kiel en la metodo de Groth, en kiu oni juĝas la efikecon de vakcino laŭ la larĝeco kaj la forteco de l'infiltracio, ankaŭ en mia eksperimento, ju pli granda estas la potenco de vakcino injektita, des pli en larĝa parto de la haŭto aperis patologia ŝanĝo kaj ofte naskiĝis variolpustulo kaj hemoragio, tial estas konvene, ke oni mezuru fortecon de reakcio laŭ la larĝeco de la papilo kaj ekzisto aŭ neekzisto de variolpustulo kaj hemoragio.

c) Se oni intencas juĝi taŭgecon de vakcino per tia metodo de inokulado, oni nepre devas jŭĝi malrekte, reciproke komparante la reakcion de la ekzamenata vakcino kun tiu de la norma vakcino, kies taŭgeco estas jam pruvita, sed ne taksi ĝin rekte nur laŭ la forteco de reakcio de la ekzamenata vakcino, ĉar individua sentemeca diferenco de kuniklo por bovinvariolaviruso influas tre rimarkinde sur la fortecon de reakcio.

Antaŭeksperimentoj II.

En tiu ĉi parto de la esplorado, unue mi observis la staton de la nasko kaj evoluo de imuneco kontraŭ bovinvariolo ĉe kunikloj surhaŭte vakcinitaj, celante scii, kiom da tempo post la komenca imuniganta vakcinado devas pasi por inokuli la vakcinon, kies potencon oni volas ekzameni. Poste, mi entreprenis determini la plej konvenajn maldensigan gradon, injektokvanton kaj tempon por observi la rezultaton. Por tiuj ĉi celoj, jenaj eksperimentoj estis faritaj.

La teknikoj kaj rezultatoj de la eksperimentoj:

1) Tiaj kunikloj kiel en la antaŭaj eksperimentoj estis uzataj.

2) Por ĉiuj eksperimentoj nur la vakcino A en la antaŭaj eksperimentoj estis uzataj.

3) La komenca imuniganta inokulado estis farata jenmaniere: la vakcino A diluita 500–one estis inokulata sur la haŭto de dorsa flanko, en vasteco de ĉirkaŭ 15 kvadratcentimetroj, laŭ la metodo de Calmette kaj Guérin. Post 24×4 horoj ĉiam formiĝis kunfluiĝantaj pustuloj.

4) Post tia komenca inokulado, ĉiutage je difinita tempo po 0.2 kubikcentimetro da vakcino A diluita milone estis enŝprucigata en la dorsohaŭto ĉiufoje nove forrazita.

5) La partoj injektitaj estis observataj ĉiutage je difinita tempo, kaj la larĝeco de la papilo estis enskribata kun ekzisto aŭ neekzisto de variolpustulo kaj hemoragio. Aldonita tabelo montras la rezultaton.

Kiel oni vidas sur la tabelo, ĉe ĉiu kuniklo, ju pli malfrue estis farata la inokulado, des pli malforta estis la reakcio, kaj samtempe des pli mallongiĝis ĝia

irado, tiel ke la inokuladoj faritaj depost la tria tago reakciigas jam rimarkinde malforte, kaj dum la inokuladoj faritaj en la unuaj 4 tagoj atingis la maksimumon de reakcio en ĉirkaŭ 96 horoj, la inokulado farita en la 5-a tago atingis tiun ĉi maksimumon plifrue ĉirkaŭ 1 tagon, t. e. proksimume 72 horojn. La inokulado farita en la 6-a tago naskis jam nenian specialan reakcion.

Dank'al tiuj ĉi eksperimentoj, mi povis ekscii, ke, por plej konvene observi la reakcion de la inokulado farita en neperfekte imunigita stado de la besto, estas bone, ke oni faru tiun ĉi duan inokuladon dum la 3-a kaj 5-a tago, kalkulante de la tago, en kiu la komenca imuniganta inokulado estis farata. Samtempe mi ekrimarkis en tiuj ĉi eksperimentoj, ke en multaj okazoj la inokuladoj faritaj en la 4-a kaj 5-a tago reakciigas nur tiel malforte, ke oni ne povas facile mezuri la larĝecon de la papilo. Dubante, ĉu tio ĉi eble dependus de la denseco de la vakcino injektita, mi preparis 5 diluaĵojn de la vakcino, en la proporcioj de 1/1000, 1/500, 1/250, 1/125, kaj 1/50, kaj inokulis enhaŭte ĉiun po 0.2 kubikcentimetro al 5 kunikloj, en la 4-a kaj 5-a tago post la komenca vakcinado, laŭ tia sama tekniko kiel la antaŭaj eksperimentoj. Jen rezultato: la pli densaj diluaĵoj kiel 1/50, 1/125 k. t. p. aperigas klaran reakcion, dum la aliaj reakciigas ankaŭ same tiel malklare, ke la mezuro de la papilo estas malfacila. Tiamaniere mi sciis, ke en tia okazo rilate densa diluaĵo estas preferinda ol pli maldensa.

Cetere, ŝajnis al mi, ke la kvanto de 0.2 kubikcentimetro estas tro multa por enŝprucigo, kaj pro tio la forteca diferenco de la reakcio laŭ diluita grado estas nuligata, tiel ke la diluaĵoj en 1/1000 kaj en 1/250 reakciigas preskaŭ samgrade. Do mi preparis 50-onan, 100-onan kaj 250-onan diluaĵojn, kaj injektis al 20 kunikloj 0.05, 0.1 kaj 0.2 kubikcentimetron el ĉiu diluaĵo en la 4-a kaj 5-a tago. Kiel antaŭsupozo, en la plej multo da okazo, la injektado je 0.05 kubikcentimetro montris la plej klaran diferencon de la reakcio laŭ la grado de maldensigo, kaj ju pli granda estis la enŝpruckvanto, des pli malklara estis tiu ĉi diferenco.

6) Jen resumo de l'eksperimentoj:

a) Se oni enhaŭte injektadas ĉiutage, dum 5–7 tagoj, saman vakcinon al unu kuniklo, des malpli forta estas la reakcio elvokita ĉe la injektita punkto, ju malpli frue estis farata la inokulado, kaj tiamaniere oni povas persekuti la evoluon de naskita imuneco kontraŭ bovinvariola viruso ĉe la besto.

b) Por povi plej klare distingi la reakciofortecan diferencon, kiu povas sin montri pro la malsameco de la potenco de kupoksvakcino, kiam oni injektas kelkajn diversfortajn vakcinojn en la haŭton de kuniklo neperfekte imunigita kontraŭ bovinvariola viruso per antaŭa vakcinado, estas necese, ke tia dua inokulado estu farata en jenaj kondiĉoj:

1) faru inokuladon post ĉirkaŭ 72 horoj, t. e. en la 4-a tago, post la komenca vakcinado; 2) diluu la vakcinon en la proporcio de 1/50. 3) ŝparu kiel eble plej la enŝpruckvanton (ekz. 0.05 kubikcentimetron).

[illegible]	sur-haŭta	enhaŭta																																		
[illegible]	la 1-a tago	la 2-a tago							la 3-a tago							la 4-a tago							la 5-a tago							la 6-a tago						
[illegible]	96	24	48	72	96	120	144	168	24	48	72	96	120	144	168	24	48	72	96	120	144	168	24	48	72	96	120	144	168	24	48	72	96	120	144	168
1	kunfluiĝanta		13.0	15.0 h p	15.0 h p	15.0 h p	....	krustiĝanta		9.0	14.0 p	14.0 p	12.0				10.0 ..	16.0 p	16.0 p	...	—			11.5	11.5	10.0	—					9.0	—			
2	"		12.0	14.0 h p	13.5 h p	13.5 h p	....	krustiĝanta		8.0	11.0 p	12.5 p	12.5 p	10.0	—		8.5	14.0 h p	15.0 h p	...	krustiĝanta			. .	11.0	10.0	—						—			
3	"		12.0	13.0 h p	13.0 h p	13.0 h p	....	krustiĝanta		7.5	11.5 p	11.5 p	11.5 p	10.0	—		10.0	13.0 p	13.0 p	13.0 p	—			10.0	10.0	10.0	—					7.0	—			
4	"		14.5	16.0 p	16.0 p	16.0 p	...	krustiĝanta		10.0	12.0 p	12.0 p	11.0 p	8.5	—		10.0 ...	14.0 p	14.0 p	13.0 p	—			12.0	12.0	11.0	10.0	—			10.0	9.5	8.0	—		
5	"		9.0 ..	10.0 p	11.5 p	11.5 p	....	—		6.0	8.0	10.0		—			6.5	11.5	15.0	15.0	kurstiĝanta					—							—			
6	"		11.0	13.2 h p	13.2 h p	14.0 h p	. .	krustiĝanta		10.0	12.0 p	13.0 p	13.0 p	10.0 p	krustiĝanta			12.0	14.0 p	11.0 p	11.5	krustiĝanta		13.0	12.0	11.0	11.5	krustiĝanta					—			
7	"		6.0	13.5 p	13.5 h p	13.5 h p	....	krustiĝanta		9.0	11.5 p	13.0 p	11.0	9.0	—		9.0	9.0	10.5	7.0	—				....	6.0	—						—			
8	"		11.5	14.5	14.5	15.0	....	krustiĝanta		10.5	11.5 p	11.5	11.5	—				12.0 h	12.0 h p	11.0	—			11.0	10.0	6.5	—						—			
9	"		12.5	16.0 h p	16.0 h p	16.0 h p	...	krustiĝanta		10.0	13.0 h p	13.0 p	12.0 p	8.0				12.0	12.0	7.0	—			12.0	13.0	8.0	—						—			
10	"		13.5	15.0 h p	15.0 h p	15.0 h p	....	krustiĝanta		10.5	11.8	13.0 h p	12.5	10.0	—		. .	11.5	12.5 h p	10.0	—			11.0	12.0	—							—			

Rimarko: 1) Ciferoj montras la largecon de la papilo en milimetro. 2) h = hemoragio; p = papulo. 3) .... montras, ke la lugeco de la papilo ne estis mezurebla [illegible] ĝia malklara konturo. 4) — montras, ke la reakcio estis tute aŭ preskaŭ tute malaperinta, kaj nun el ĝi estas kovritaj de makuleta "Skvameto", sed ne vera krusto.

c) Estas plej konvene, ke oni observu la rezultatojn je la 96-a horo post la dua inokulado, ĉar la reakcio de la inokulado farita en la 4-a tago atingis kutime la maksimumon en tiu ĉi horo. (vidu la tabelon).

La ĉefeksperimentoj.

En la printempo de la jaro 1921, mi inokulis en la haŭton de kunikloj imunigitaj neperfekte, 38 specimenojn de vakcino, nekonata pri ĝia potenco, uzante la vakcinon A suprediritan, kiel norman vakcinon, plenigante ĉiujn necesajn kondiĉojn suprediritajn. Ĉiu specimeno estis nepre injektata al 3 kunikloj, kaj ĉe ciu besto estis inokulataj 5–6 specimenoj samtempe. La reakcio de la ekzamenata vakcino estis precize observata, komparante kun tiu de la norma vakcino. Iuj el la unuaj reakciigis pli, iuj malpli forte ol la norma, kaj aliaj estis samaj kiel tiu ĉi. Poste, ĉiuj specimenoj, taksitaj pri sia potenco inokulante al kunikloj tiamaniere, estis inokulataj al 380 Tokiaj geknaboj reinokulotaj (al po 10 personoj ĉiun specimenon), per tiel nomata krucentranĉmetodo, kaj la rezultatoj estis observataj post 8 tagoj. Bedaŭrinde en tiu ĉi eksperimento, mi ne povis kompari la potencon de kelkaj specimenoj kun tiu de la norma vakcino, ĉar multaj el la inokulitoj jam en la jaroj 1916–7 estis vakcinitaj eksterperiode pro tiama variola epidemio en la urbo, kaj konsekvence ne estis malmultaj tiaj individuoj, ĉe kiuj montriĝis nenia reakcio speciala per la ekzamenata, nek per la norma vakcino. Tamen, koncerne al la aliaj, ilia potenca diferenco de la norma vakcino entute koincidis kun la rezultatoj observitaj en la antaŭaj kuniklaj ekzamenoj.

Por pli konfirmi la taŭgecon de mia metodo, en la aŭtuno de la sama jaro (ĉiuj vakcinoj estis konservataj en glacitenejo de ĉirkaŭ −5°C.) mi kontrolis per la metodo de Kii, uzante 5 bovidojn, la specimenojn, kies potencon oni povis taksi per la homaj eksperimentoj ĉisuprediritaj, kaj komparis la rezultatojn kun tiuj de la homaj kaj la kuniklaj ekzamenoj. Mi vidis, ke la rezultatoj tute koincidas ne sole kun tiu de l'homoj, sed ankaŭ de la kuniklaj ekzamenoj. Tial mi povas diri, ke la rezultatoj de la kuniklaj ekzamenoj tute ne estis hazardaj trafoj.

La Konkuldo.

Oni povas kontroli tre ekzakte la efikecon de kupoksvakcino, se oni enŝprucigas ĝin samtempe kun la vakcino, kies taŭgeco jam estas pruvita, en la haŭtohiston de kuniklo imunigita neperfekte kontraŭ bovinvariolo per la vakcinado farita antaŭ kelkaj tagoj, kaj komparas reciproke la fortecon de la reakcio elvokita ĉe la injektita punkto de la ekzamenata kaj la norma vakcinoj. Kaj tiu ĉi metodo superas unuflanke la metodon de Kii, per sia facila operacioteknikо kaj malgranda elspezo, aliflanke la metodon de Groth, ĉar laŭ mia metodo oni povas ekzameni pli multajn specimenojn de vakcino uzante malmultajn bestojn.

Pathology

On the Function of the Lymphocyte.

By

Prof. **Mataro Nagayo,** Director of the Institute.

Lecture read before the General Meeting of the Kiushu Medical Congress on Nov. 11th. 1922.

For many years, I have observed with interest the function of the lymphocytes and have had my assistants investigate along this line both in the Government Institute for Infectious Diseases and in the Pathological Institute of the Tokyo Imperial University. The results of these experiments form the basis of my argument.

The numerical proportion of the lymphocytes and myelogenous leucocytes in blood differs according to the animals, for example in the cat and the dog the lymphocytes are found to be 15–25% of the total leucocyte count while in many laboratory animals, i. g. guinea-pig or rabbit, they average 45–50% or more. In man they vary according to the age, in suckling more than 50%, but decrease numerically until approach adult life. In adult the numerical ratio of the lymphocytes and the leucocytes is generally regarded as 1 : 2.2.

According to *Tatara*'s investigation, such a ratio is found generally at the age of 18 years, i. e. during the age of adolescence, and bears a certain relation to the regressive metamorphosis of the thymus gland.

With regard to the large lymphocytes, there are many different opinions, some regard them as a regressive form, others as a young form of the lymphocytes. *Naegeli* said that it is impossible to determine the age of cells by their size, but only by the structure of the nucleus. The majority of the large lymphocytes are young cells and indicate regenerative process going on in lymphatic organs. *Okada* has made many investigations along this line, clinically on typhoid and tuberculouse patients and experimentally on rabbits vaccinated with typhoid vaccin or autolysate and emulsion of lymph gland. In the course of some infectious diseases like typhoid, there is a remarkable numberical change of the lymphocytes, mostly an initial lymphopenia with increasing fever which arrives at the highest point at the height of the disease and a lymphocytosis with decreasing fever which is found in convalescence, as a postinfectious lymphocytosis.

On the other hand, in some diseases, an initial lymphocytosis is seen, for example, in whooping cough, chickenpox and dysentery.

In some protozoan diseases there is also seen lymphocytosis, for example in trypanosomiasis and in kala-azar, *Mitsuda* found that in leprosy of nervous system a pretty strong lymphocytosis is seen, some times averaging 50% of the total leucocytes, contrary to this in nodular leprosy there is usually a lymphopenia. In the experimental rice neuritis of chicken *Ogata* found that in the initial stage showing hyperglycaemia in blood and no nervous symptoms, a remarkable lymphopenia was observed bearing no relation to starvation.

It is a well known fact that the starvation causes atrophy of the thymus gland and is accompanied by lymphopenia. Further investigation of *Ikeda* found a momentary lymphocytosis in blood and a certain regressive change in the lymphatic organ with a few karyokinetic figures. With progressing of starvation the animal shows a remarkable lymphopenia and severe regressive metamorphosis in the lymphatic apparatus. If by this time one lets the starved animal take food, its lymphatic apparatus shows immediately a regenerative process with lymphocytes increased in number in the blood. By the experimental study of starvation on the animals, it is quite evident that mobilization and consumption of body fat bear a certain relationship to the lymphocytes in blood and the changes of the lymphatic organs, i. e. there is a certain relation between the metabolism of body fat and the function of the lymphocytes.

The lymphotoxin obtained by immunization with the lymphocytes has a specific affinity for the lymphatic system, causing degeneration in lymphatic organ and lymphopenia in blood, when it is inoculated into the responsed animals, *Yokomori* has made various experiments along this line, he injected into rabbits an emulsion of hetrogeneous or homogenous lymphocytes or with their autolysate and found a slight lymphocytosis or a remarkable lymphopenia according to the dose injected, the latter of which especially strong on the next day, and nearly no change in spleen, bone-marrow, erythrocytes and myelogenous leucocytes. *Okada* has made further studies upon the same subject and confirmed the *Yokomori*'s results with other new findings.

They found the specifity of the lymphotoxin and the direct action of the components of dead cells. *Mita* and *Miyagawa* have communicated recently a new theory on this point, that the components of dead cells act upon and degenerate the homologous tissue or organ. It is not yet quite settled that the cause of the degeneration of the lymphatic system occurred by injection of emulsion of lymphocytes is of cytotoxic nature (*Mita*) or of the direct action of the cell components (*Miyagawa*). The degeneration caused dy injection of emulsion of lymphocytes consists of regressive metamorphosis with a slight regeneration of lymphocytes but in severe case only of regressive, at that time a lymphopenia in blood and moblization at body fat are seen just like as that of the starvation of animals.

As *Sternberg* and *others* have mentioned, in some chronic infectious diseases, especially caused by a certain bacterial germs or protozoa, the lymphocytes and

the plasma cells gather in the focus of the disease, for example, in tuberculosis, syphilis, actimomycosis (in the surrounding of abscess), rabies, mycosis fungoides, Blastomycosis infection, gonorrhoea, dysentery, spring catarrh, trachom, etc. In certain diseases of central nervous system i. g. in dementia paralytica, sleeping sickness, poliomyelitis anterior acuta, rabies, encephalitis lethargica, typhus, chicken pox and Borna disease, these is quite characteristic of infiltration of these cells, appearing in the lymphatic spaces surrounding the blood vessels.

What is the cause of such a phenomenon?

It is not quite settled that the lymphocytes might have some affinity for certain germs or toxins.

In acute inflammation, the infiltration of the myelogenous leucocytes primarily occurs at the focus of the disease and secondarily that of the lymphocytes. In non-infectious diseases there is also very often the infiltration of various kinds of cells in a characteristic manner, for example, the infiltration of polynuclear cells occurs close the necrotic tissue and that of the lymphocytes a little more apart from it in the spontaneous gangren of a finger or toe as in a protective group. According to *Homma*'s experiment, a dead parasite or muscle tissue inoculated into the subcutaneous tissue of a animal are invaded at first by the neutrophilic leucocytes, surrounding the strange corpuscles and secondarily by the eosinophilic leucocytes and lymphocytes, which are working in order to absorb the foreign bodies.

From the above described facts, it is clear that the lymphocytes have a certain preventive action against bacteria or toxin.

Meyelogenous leucocytes may contain a certain proteolytic ferment and secrete it according to need. In lymphocytes there may be a kind of lipase which decomposes fat and carbo-hydrate.

But such an idea is not as yet recognised by all authors. More recently *Bergel* described that the lymphocytes contain a kind of lipase from the following facts; (1) the emulsion contained the lymphocytes is able to decompose and dissolve yellow phosphorus (2) if a glass canula filled with fat is placed in the peritoneal cavity of an animal, the fat at the end of the canula is decomposed, making a fatty acid and there are seen a large number of lymphocytes. (3) if fatty oil is injected into the peritoneal cavity of an animal, myelogenous leucocytes at first and then lymphocytes are coming out by amoeboid movement, in order to take the oil and to digest it, (4) if the erythrocytes are injected into the peritoneal cavity the lymphocytes gather about them in order to dissolve the lipoid capsule of the erythrocytes.

But from the histological point of view, *Bergel*'s description is not correct. For example, he considered presumably the histiocytes as the lymphocytes, that *Aschoff* and *Kamiya*, have already pointed out. According to *Nishibe*'s experiment the lymphocytes gather relatively few in number every time of the same experiments as those described above by *Bergel*, and they take never what in.

Caro studied the content of lipase in blood serum following various kinds of diseases and found no relationship between its content and the number of the lymphocytes in blood and showed that the content of lipase in the emulsion of lymphogland was not more than that of the serum. These facts are also contrary to *Bergel*'s findings.

At any rate, from *Ikeda*'s and *Yokomori*'s experimental results I like to think that a certain relation may exist between the function of the lymphocytes and the metabolism of fat, but it does not mean that the lymphocytes would have a high content of lipase.

For many years I have been interested to know, "where are" the lymphocytes performing their function and "what is" their destiny.

It is a well known fact that leucocytes and lymphocytes are found in the oral cavity as the so-called salivary corpuscles and that leucocytes escape through the intestinal wall. These facts, especially the functions of lymphocytes in the intestinal canal have not been written about by any physiologist and also have not been studied by any particular investigation. *Bunting* has communicated recently in a some journals, that lymphocytes emigrate out through the intestinal wall into the cavity generally in a suprisingly large number and hence lymphocytes probably have a certain function on the surface of the digestive tract. A few years ago I took notice of this fact, that the so-called round cells in the submucouse membrane of the intestine are different from each other in their source or in number according to the part of the intestine, to the age of the person and to the type of disease.

Since then I have found very often the lymphocytes emigrating out through the intestinal wall into the cavity intermingled with other round cells, but I have paid little attention to their biological significance. Recently I have had many opportunities to examine the animal's tissue under microscope and have been obliged to take notice of the above described phenomenons and finally I have arrived at the conclusion that the lymphocytes emigrated into the intestinal cavity should have a certain biological function. I would like describe the basis of my argument as follows:

1. How large a number of lymphocytes emigrate into the intestinal cavity?

In order to determine the number of the emigrating lymphocytes, it is the best to examine the section of animal tissue or the smear of the intestinal contents, by which a surprisingly large number of lymphocytes are found between the epithelial cells or intermingled with its content. It seems that in a guinea-pig the lymphocytes are daily coming out through the intestinal wall probably in a number of many hundred million, which is therefore incomparably larger in number than that of other leucocytes emigrated out through the intestinal wall.

2. Numerical difference of emigrating lymphocytes according to the locus of the intestine.

In the mouth the lymphocytes come out most abundantly from the tonsils and the follicles in the root of the tongue, very few, or nearly none, from the oesophagus. Generally speaking the lymphocytes come out in very few numbers through the stomach wall, especially few from gastric body and gastric fundus, on the contrary more or less large number from the pyloric region.

In the intestine they come out most abundantly from the duodenum and the large intestine, especially from the ileo-coecal part, the appendix and from the coecum, very few from the ileum and the terminale portion of the large intestine, in the jejunum in a increasing number from the upper to the lower part.

3. Morphology of the emigrated lymphocyte.

From the morphological point of view, the eliminated lymphocytes show no regressive metamorphosis, until the end of their emigration between the epithelial cells. On the contrary, the already emigrated ones dissolved and perished immediately after their work. Lymphocytes make no amaeboid movement, but have a certain relation to automatic movements. If the lymphocytes had been already degenerated at the time of their emigration, it would be impossible for them to come out through the intestine wall. From this view point, the emigrating cells are quite normal and healthy, and their decadence takes place after their passing out.

4. The position of the lymphocytic organ in the intestinal wall.

The lymph-follicles are generally situated directly beneath of the epithelial cells, according to the part of the intestine, its structure is different from each other, i. g. the appendix has a characteristic structure, having the lymph-follicles quite closely attached to the epithelial layer and consequently having only a small amount of loose connective tissue between them.

According to *Takenouchi*'s experimental result, the epithelial cells covering the lymph-follicles have a specific and different property from those of other parts (Resportion of indianink). The majority of lymphocytes made in the lymph-follicles emigrate directly into the intestinal cavity through the space between the covering epithelial cells and a few of them come down into the lymph spaces, then into the lymph vessels, by which they arrive at the mesenterial lymphsystem. From this point of view, it is clear that the lymphocytes in the lymph-follicles or glands in the intestine are coming into the intestinal cavity in order to perform there a certain function.

5. From the stand point of the comparative anatomy.

The man and other mammals have a large amount of lymphatic tissue, some of it as the lymph glands distributed in various kinds of organs or tissues. In feathered tripe, reptilians, amphibians and fish (as Katsunuma said) there are only the spleen follicles and no lymph glands, with the exception of a certain number of lymph tissue in the intestine wall. This fact shows that the lymph apparatus is distributed much more in the intestine wall than in other part.

6. Why are they necessary in the intestine wall?

They lymph apparatus is generally recognized as a kind of preventive arrangement in an organism, for example againt any kind of germs or toxins. From this view point, I may say that to have such an apparatus in the intestinal wall would be rather dangerous, more inconvenient and not profitable at all, because most kinds of infectious germs, i. g. of typhoid, cholera, dysentery and tuberculosis invade usually the lymph-follicles first and rather seldom other normal mucous membranes, just the same in the lacune of the tonsils. It is therefore quite clear that the lymph apparatus has a much more important rôle than that as a preventive measurement, not only sending the lymphocytes made in it to the blood vessels, but also a much more large number of them into the intestinal cavity in order to maintain their important biological function.

From the above described facts, I recognize that one of the functions of lymphocytes existe in the intracavity of the intestine. What is the function, for example, of the digestive process, against a certain toxin or of some other vital phenomenon? It is not clear up to the present time, and it needs further studies along this line. At any rate, the lymphocytes in blood or in the intestinal follicles emigrate for the most part into the intestinal cavity, that means not merely an excretion of the lymphocytes, rather a biological significance on account of doing a certain vital mission. If my idea were correct, then a certain pathological change dealing with lymphocytes would be easily explained. I will take as an example lymphopenia or lymphocytosis. The number of lymyhocytes in circulating blood depends upon the regulation of the excretion of the lymphocytes into the intestinal cavity and of the production from the lymph apparatus. If we found lymphopeny or lymphocytosis as a symptom of a certain disease. i. g. typhoid, dysentery, lymphatic leucaemia or Hodkin's disease, we should therefore search the orgin of the symptom always considering two points, namely the disturbance of excretion and production of lymphocytes.

From this point of view, I think, a new sphere for research concerning the lymphatic function or change in an organism, exists in our medical science. I have let my students work along this line, and hope to make further reports in the future.

The Organ or Tissue Cells as an Irritant or Toxin and the Procedure of the Creation of Organ Toxin.

By

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What is the main process of the life of mammals? We know that the food is taken in through the mouth, and digested and absorbed in the alimentary canal; the proteids becomes the tissue of the body; carbo-hydrates and fat generate the heat of the living body by oxidation and combustion, or transformed into energy doing all kinds of work; the movement of the heart makes the blood circurate through the whole body; the respiratory movements of the lungs inhale oxygen and exhale carbonic acid gas; the urine formed in the kidneys is excreted by the contraction of the bladder; the residue of the undigested food is conveyed out of the body by the movement of the intestines. To the functions of these various important organs of the body we may add the movements of the limbs by means of which its various desires are satisfied. We know that it is by the functioning of these organs that the process of life in all its phases goes on. But by what irritation is the specific function of each organ performed, and by what are these complicated functions properly regulated? This is an important and difficult problem. It would be too unscientific to dismiss it by saying simply that it is all due to the instinct of life to attain the aim of its pursuit. Some might say that such a control and regulation belong to the function of the central nervous system. But is it due solely to the action of the central nervous system? That is the question. There is no doubt that the central nervous system governs and regulates the functions of the various organs. But is there not any other natural regulator? To express my view on this latter point is the object of this dissertation.

What action have the components of dead cells upon the living body?

From the early times, the study of metabolism has engaged the attention of investigators, and there have been many considerations or investigations as to

how the material derived from outside is assimilated into the living body. There have been many investigations concerning the destination of proteids, fat and carbo-hydrates after they are taken into the body as food, and also concerning the manner in which they are assimilated. But investigations concerning the effect that the assimilated matter will have on the body, when it is dissimilated and excreted into the outer world are by no means so numerous. Far fewer are the investigations as to how the components of cells which are the elements of our body are disposed of when it is dead, or what action they will have upon our body. As the matter of fact, the cells of the human body and of any living body are constantly breaking down in the physiological or normal states, and their components are constantly being eliminated out from the body. They replace the loss by taking and assimilating daily food. Therefore, when the tissue of a certain organ is affected with a disease, it can easily be imagined that the breaking down of cells will be unusually large in that part. The components of these dead cells, no matter whether it is of physiological or pathological origin, either through circulation, or directly, through there spiratory organ (like sputum), or through the excretory organs (like urine or faeces), or through the skin of the body, or mixing with various kinds of excretions and secretions, are eliminated from the body. If the components of the dead cells are absorbed into the blood circulation, will they not cause harm or disturbance? This is an important question which has been rather neglected by old scholars. Many parenchymatous cells of the lungs break down in acute pneumonia. But all of these components of dead cells are not ejected together with the sputum. It is a well known fact that a part or the most part of them are absorbed by proteolysis and carried away by the blood circulation. Whether the components of the parenchymatous cells of lungs could be eliminated from the body without having any action at all upon the body is a serious question which no one yet has thought of. Similarly, the cells of the lung tissue are constantly breaking down in the physiological process of the normal body, and their components are absorbed into the sputum or the blood circulation just as in the case of pneumonia. The action of the components of dead cells in this case is also an unsolved problem. But if there is any action at all, it must be of the same kind as that which is associated with the pathological case above described; only the action would be much weaker. For instance, take the case of the gastric and intestinal glands. Suppose, on account of various diseases, many of the cells of mucous membrane of the digestive tract die at once. To think that these dead cells will all be digested or fall off from the wall of the digestive tract and be merged with their contents would be looking at only one side of the shield. Surely a portion of the components of these dead cells will be absorbed in varying quantities and pass into the blood circulation. A similar phenomenon but in a lesser degree in always present in the normal body. Accordingly the action in the latter case differs only in the degree. I have been able to ascertain from my various experimental investigations

that the action of parenchymatous cells which is manifested when they are absorbed and pass into the blood circulation, is very complicated and important, no matter whether they were originated physiologically or pathologically. As the result of my investigation, I discovered the following fact, "*If the tissue or organ cells are parenterally (not through the mouth) injected into the organism, they act upon the homologous tissue or organ cells.*" In other terms, we may say that if the components of the parenchymatous cells of lungs are absorbed, they are circulate in the blood vessels act upon other normal parenchymatous cells of the lungs, and in the same way the components of the cells of the mucous membrane of the digestive tract will pass into the blood circulation and act upon the other normal cells of mucous membrane of the digestive tract; and if the action is not too strong, it will stimulate the homologous living cell and will maintain the physiological function of the organism, and if it is fairly strong it will excite the function in an unusual degree, and if it is too strong, it will have the effect of actually destroying the cells.

Before I proceed, let me say a few words about how many cells are dying in the physiological or normal states of the human body, or how the metabolism of cells is going on.

The number of cells (e. g. red blood corpuscles) perishing in the living body.

The cells constituting our living body are not invested with everlasting life. We all know that they are perishing each moment and are being replaced by new cells. The period of life, of course, differs according to the kind of cells. The cells of liver, kidney, lung, nerve cells, muscle cells, nerve fibre and connective tissue may have all their appropriate length of life. But as it is exceedingly difficult to determine them, I shall try to state my view concerning the red blood corpuscles which are relatively easy to calculate.

The total quantity of blood.

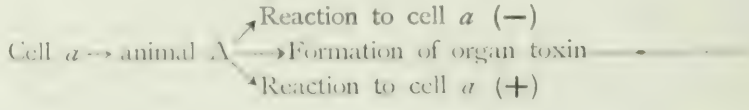
As to the question what is the total quantity of the blood of the human body, the different figures of 1/13 or 1/15 or 1/17 of the weight of the body are given. For the sake of calculation I shall choose 1/15 of the weight of the body as the total quantity of our blood. There have been several investigations concerning the days of life of the red blood corpuscles. Usually, the life period is said to be 3 to 4 weeks. Some would give 30 days at longest. Others would make it still longer. But I might take 30 days as the period of the life of red blood corpuscles. I count their number as about 5,000,000 in one cubic mm, and fix their specific gravity as 1055 or 1060. Thus I get the following figures.

(1) The weight of body (human being) 50 kg.
(2) The number of red blood corpuscles. in 1 c.mm 5,000,000.
(3) Days of life of red blood corpuscles 30 days.
(4) Specific gravity 1055.
(5) Total weight of blood 1/15 weight of the body $1/15 \times 50 = 3{,}333$ kg. $3{,}333 \div 1055 = 3159{,}5$ c.c. = 3160 c.c. = nearly 3,160,000 c.mm.
(6) The number of red blood corpuscles perishing in a day. (in 1 c.mm.) 5.000.000 ÷ 30 = 17,000 nearly
(7) The total number of red blood corpuscles perishing daily $17{,}000 \times 3{,}160{,}000 = 537{,}200{,}000{,}000$
(8) Counting the number of red blood corpuscles in 1 c.mm as 5,000,000 and calculating the number of perishing red blood corpuscles in terms of c.mm we get $537{,}200 \div 5 = 107{,}440$ c.mm = 107 c.c. nearly.

The number of red blood corpuscles perishing in a day in a man of 50 kg. corresponds to that contained in 100 c.c., a surprising number!

According to this calculation, the number of red blood corpuscles correspond to that contained in 5 c.c. daily break down in a rabbit weighing 2,5 kg. There are many other modes of calculation. But the one described above may be sufficient for the purpose. My purpose being to know how the components of these dead cells are utilized in the living body, let me speak now about the red blood corpuscles. It is well known that the blood pigment becomes the component of the bile. But, it should not be supposed that the other components are eliminated from the body without further utility. On the contrary, a large quantity of these ruined components is ingeniously utilized in the life of the human body, especially in its physiological functioning. When their irritation is strong, they quicken the function, and act on the hematopoetic organs, accelerating the regeneration of the red blood corpuscles. Finally when they are too strong, they go beyond the threshold of irritation and cause the destruction of normal blood corpuscles and consequently anaemia. Dr. *Ono* has made an investigation in this line on the basis of my suggestion.

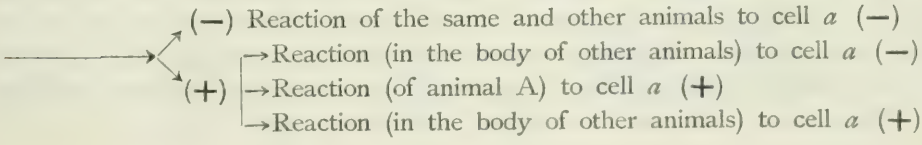
The organ or tissue cells as an irritant or toxin. From the above explanations, my readers may perhaps have imagined or even understood to some extent, what I want to say. But let me neverthless repeat my theory once more and try to make it as clear as possible. The thesis is, "*if the organ or tissue cells are parenterally, (not through the digestive tract) injected into the organism they act upon the homologous tissue or organ cells, and the organ toxin corresponding to the injected cells is generally created in the organism.*"



When cell *a* is parenterally injected into an animal A, it reacts not only to cell *a* but also to the cells of other organ or tissue in various ways, (although the reactions in this case are not definite); and when the reaction of cell *a* does not appear, or is very slight, the reaction to the cells of other organs or tissues either does not appear or is also very slight; but sometimes the reaction to cell *a* alone may take place. There are only two ways of examining such reactions, namely, the observation of changes in the function of cell *a* in the animal A, and making the histological examination by dissecting the animal. When the quantity of injected cells is small, we notice the quickening of functionating and the histological changes of varying degrees are recognized. In such a case, it is quite reasonable to expect that there should often be various changes, in other organs. It came to my notice for the first time that when the components of cell *a* are absorbed and enter the blood circulation, they act on the surviving cell *a*. Such action is always going on in ordinary physiological states. But when a tissue or an organ is affected with an disease and an unusually large number of cells break down and perish, the components of these dead cells will be absorbed in an unusual concentration and will give the homologous or heterologous cells the supra-physiological irritation, and cause the progress of the disease or complete the various forms of such disease.

If the quantity of the absorbed components of dead cells is not great and their action does not amount to more than physiological irritation, the function of the organ concerned will be maintained in good physiological order. When the action is fairly strong, it will quicken the functioning. It is true that the irritation which enables each individual cell of the normal body to perform its physiological function depends upon the regulation of the nervous system. But it is my belief that the action of the components of cells broken down and absorbed upon the survived homologous cells is by no means insignificant, and it is by the direct irritation of this action that the normal cells perform their function. *I shall call this action by a specific term viz. the* **direct action** *of the cell component, and would distinguish it entirely from the action of the immune body produced by immunization.*

Now I shall try to explain what I mean by the term direct action. I have already stated that the cells in our living body (no matter of what kind, or no matter in what physiological or pathological states they may be) are always dying and breaking down. A portion of the components of the dead cells is directly eliminated from the body, by means of the digestive organs, the excretory organs, the respiratory organ or the cutaneous tissue of the body, and has no



reaction to the living body. But the other portion is absorbed into the blood circulation, and it is absorbed in an unsually large quantity or in a non-physiological state in pathological cases. This absorption may be regarded the same in effect as the parenteral injection of the cells into the body and their subsequent passage into the blood circulation. And it can be easily imagined that the cell components thus absorbed, like those which were observed in my investigation, would produce the action on the homologous cells. The direct action going on in the living body even in the physiological state, is so weak as to produce just enough irritation as to enable the cells to maintain their physiological function. But if on account of a certain disease, the number of dead cells become much more larger than in the ordinary physiological process, then the direct action becomes too strong and brings forth a pathological phenomenon. It is not difficult to prove this kind of direct action experimentally. Many experiments made under my supervision have indeed done so. For instance, a disturbance of the kidney experimentally produced on one side, may produce a reaction on the normal kidney of the other side (*Kimura*). When we cause the formation of an ulcer in a part of the mucous membrane of the stomach, we can produce various histological changes in the mucous membrane of the other part of the stomach which has no relation to the affected part. (*Miyagawa, Murai, Terada*). Again, when a part of the liver is crushed or a subcapsular injection of hepatoxin is made, the functional change in the liver is noticed. (Miyagawa and others). If one of the testicles is crushed or affected with various kinds of diseases, the other normal testicle atrophies, for the crushed or dead cells entering the blood circulation, brings direct action to bear too strongly upon the cells of the normal testicle. In such a case, the removal of the diseased testicle alone would bring about the hyertrophy of the normal testicle. (*Miyagawa* and others). These instances would show how the components of dead cells in the living body have a direct action upon itself. Abundant literature concerning what may be regarded as direct action is not wanting.

The instances in which cell *a* of animal B is injected into animal A.

I shall now turn to the changes produced by the injection of cell *a* of animal B into animal A.

The liver. The liver of a rabbit is taken out, crushed and ground up well, and an emulsion is made out of it. The intraperitoneal or sub-cutaneous injection of this emulsion into an other normal rabbit is made. When the quantity of injection is more than one gram per kilogram of the weight of the body, the liver of the animal shows a change of high degree. Often necrotic patches of various sizes are seen, and there is an extreme decrease of the glycogen content. As the quantity of the injection is gradually decreased, the histological change also decreases, and we can so regulate the change that it passes from the

disturbance to the quickening of functionating. This fact became clear by the investigation of our co-workers. Then I had *Kodama* make the following experiment. The biliary fistula of the gall bladder was experimentally formed in a dog, so that the bile issued to the abdominal wall.

The emulsion of the liver of a rabbit was injected into the peritoneal cavity of the dog, and the state of the bile-secreting function of the liver was examined. The use of 2 gram, 1 gram, and 0.5 gram of emulsion for every one kilogram of the weight of the body each caused a functional disturbance of the liver, and an extreme decrease in the quantity of the bile. The autopsy of the animal disclosed necrotic patches here and there in the liver. But when from 0.01 to 0.05 of the liver emulsion was injected, the secretion of bile was quickened and the amount of various kinds of ferments contained in it increased. Now these two experiments may be explaned in the following manner. The components of the cells of liver are abosrbed and act upon the homologous cells of the liver. If the quantity of emulsion injected is too much, the irritation goes beyond the physiological threshold of irritation, and the degeneration and necrosis of cells follow. But if the quantity is not too much and the irritation is moderate, the quickening of functionating results.

The kidney. The experiment of *Kimura* made under my direction was nearly the same. When the intraperitoneal injection of the emulsion of the kidney of cows, horses, rabbits, guinea-pigs was made at the rate of from one to two grams per kilogram of the weight of the body, various forms of parenchymatous nephritis appeared. When the quantity of emulsion was decreased in the descending order, the irritation gradually decreased until it could be brought within the the threshold of irritation. The experiment on the testicle made by *Iida* ended in a similar result. The autopsic appearance of the cells of the mucous membrane of the digestive tract observed by *Murai* perfectly agreed with those that have been described. The result of the observation of the adrenal body, and lungs by our co-workers was such as endorse my assertion. The appearance of the central nervous system is very interesting. But I shall refrain from entering this subject. The autopsical appearance which *Yokomori* obtained by injecting the emulsion of the tissue of the lymph gland of rabbits and other animals belongs also to the direct action. This is confirmed by the investigation of lymphocytes made by *Okada* under my supervision. The pathological change caused by the injection of the emulsion of the cells of the lymph gland in varying degrees must be regarded as the result of the direct action of the injected components of lymphocytes. In a word, the action produced when the components of cells which have perished in the living body, and absorbed into the blood circulation, is essentially the same as the action which is produced by the parenteral injection of the homologous cells. A minute description of each instance is hardly necessary, but for the sake of absolute clearness, we may perhaps cite, one or two cases.

Action of the components of the red blood corpuscles upon the living red blood corpuscles.

I have already stated what an important part the red blood corpuscles play in the vital function of the human body. I have also stated my computation as to how many red blood corpuscles daily perish in the living body. But how is their function governed? The individual red blood corpuscles have no direct relation with the nervous system. It is the function of the heart that makes them circulate through the blood vessels, flowing from the left to the right, that is, flowing from the region of high blood pressure to that of the low pressure, just as water moves from the higher to the lower places. As the red blood corpuscles have no nuclei, they are regarded by some as a sort of a lumppf proteids. But they are really individual cells having a life of their own, and discharging an important duty by their metabolism. The question whether or not the function of red blood corpuscles is entirely mechanical or chemical and receives no biological irritation is an important and unsettled one. Combining the autopsical apperance observed by *Ono*, who made the investigation under my direction, and the results of the investigations of a few others, I shall write my view concerning this problem. By frequent subcutaneous injections of a very small amount of a blood toxin called phenyle hydratin into the rabbit, we can cause a very severe anaemia in the animal. If at this time, the blood taken out of the blood vessel of the same or same kind of animal and caused entirely to perished, is injected into the blood vessel or the peritoneal cavity or under the skin, the animal recovers from anaemia rather quickly or fairly well. If too large an injection is made, the number of blood corpuscles will make a considerable decrease for a time and then make an increase. The recovery from anaemia means not only the increase of the number of blood corpuscles, but also the increase of the hemoglobin. In other words, the function of regenerating the red blood corpuscles is quickened, and consequently the function of the blood corpuscles is quickened. Then the index of pigment approaches the normal state. A similar experiment can be made by causing anaemia in animal by artificial bleeding, and injecting the blood which is taken out of the animal and which has completely perished. The appearances thus obtained are nearly alike. Hence we can imagine that the component of the red blood corpuscles parenterally injected, acting on the hematopoetic organs, quickens the regeneration and excites the function of the red blood corpuscles. Turning now to the normal body, if I am not mistaken in my calculation, a surprisingly large number of red blood corpuscles are perishing daily. That the component of these perishing red blood corpuscles would have no biologcal significance is a view I can nover approve. I believe that the components of these dead cells in the manner described in the above experiment have what I have termed direct action upon the surviving red blood corpuscles and further extend its action to the haematopoietic organ, quickening the process of regeneration.

Is it not because of this direct action that the red blood corpuscles which have no relation to the nervous system can perform their important duty and regulate their function according to need?

The respiratory organ.

Let me now consider the respiratory process. The repiratory organ comprises the lungs and the upper respiratory tract connected with them. But the main part is, of course, the lungs. The respiratory movements of mammals are made when the thoracic cavity automatically enlarges or diminishes by the contraction or relaxation of the respiratory muscles of the thorax, the abdominal wall or diaphragm, and the lungs in response to these movements, simultaneously, contract or relax by the muscular action of the bronchial wall. The external air is inhaled and sent into the alveoli by the contraction of the thorax. While we can make such movements consciously (as in the case of deep breathing), we can do it also unconsciously and instinctively (as in the case of respiration in sleep). Sometimes they are done reflexively, as in the case of coughing. But the most physiological and natural are the conscious and unconscious respiratory movements, especially the latter. The respiratory movements are governed by the respiratory centre of the medulla oblongata, which on receiving an irritation of a certain strength causes the movements in the respiratory muscle and produces the repiratory movements. The physiological irritation of this nervous centre is the lack of oxygen and the increase of carbonic acid gas in the blood. When the blood laden with carbonic acid gas passes the part of that nervous centre it automatically excites the centre and produces the respiratory movements. The air enters the alveoli by the repiratory movements, and the oxygen in the air, by combining with haemoglobin flowing in the capillary blood vessels around the wall of the alveoli, is changed to oxyhemoglobin. The carbonic acid gas in the capillary blood vessels is eliminated into the air in the alveoli, perhaps by some physical (or chemical?) action. In this way, a perfect gaseous exchange is made and the dark colored venous blood becomes the scarlet arterial blood. This is the phenomenon of respiration which is constantly going on in the living body. From what has been described it seems that the respiratory mechanism is perfecly explained. But a closer examination will show that there still remain many points which are not clear. For instance, there is the question, what function has the epithelial cells which cover the surface of the alveoli (which are the parenchymatous cells of lung tissue)? This is an important but hitherto unnoticed problem. That the exchange between the oxygen and the carbonic acid gas takes place by diffusion or by some chemical action between the external air and the components of cells in the living body is the view agreed upon practically by all scholars. If so, the nature and function of the epithelial cells on the surface of the aveoli must have an important rôle for respiration, and it can be easily imagined that, when the function of that cell goes on vigorously, the gaseous

exchange that takes place there should also be flourishing, and the oxygen content in the blood should be consequently increased. What a great significance the quantity of the oxygen content in the blood has for the life of our body hardly needs to be said. Various kinds of food especially carbo-hydrate and fat, in order to become heat and energy must be oxidized. In various diseases, e. g. in tuberculosis, on account of the poverty of the oxygen content in the blood, the patient looks anaemic and pale. This is due to the destruction of the lung tissue by the focus of tuberculosis. But the toxin of the tubercle bacillus may also cause anaemia. If in such cases, the quickened functionating of the alveolar epithelial cells could be secured by some method, the usefulness and sphere of the application of such a method will be very great. But up to this present time we have not known how to quicken the function of the alveolar epithelial cells, by physiological and artificial methods. After several experiments in this line, I have come to the following view.

If a given amount of well-ground lung tissue of a rabbit is injected intraperitoneally or sub-cutaneously into an other normal rabbit, the lung, especially the alveolar epithelial cells of the injected animal shows a symptom of irritation. If the quantity is more than one gram for every one kilogram of the weight of the body, the epithelial cells cannot stand the irritation and will show various pathological changes; the cells may die and the part shows certain pneumonic symptom. If the quantity is gradually decreased and brought to the adequate quantity, the epithelial cell may be irritated and its function be quickened, but shows no pathological change. When the emulsion of the lung cells of an animal is injected into an other normal animal, the part which undergoes the most remarkable change is the lung, especially its epithelial cells. There is not usually such a violent change in other organs as in the lung. Some of my readers may think that the intraperitoneal or subcutaneous injection of the lung tissue of other animal is too unusual or too violent a manipulation. But the subcutaneous injection of a given small quantity of it is by no means a violent manipulation. As I have often stated, the object of such a manipulation is simply to absorb the components of the alveolar epithelial cells into the blood vessel. The tissue of our lungs is constantly breaking down and a portion of its components is eliminated with the sputum, and the other portion may pass into the blood circulation. It is not difficult to imagine that the component of the lung tissue, absorbed in the blood vessel in the above-described experiment would act upon the epithelial cell. If a part of the lung is affected with a disease its parenchymatous cells will break down in varying quantities according to the state of the disease. A portion of their components will be absorbed and irritate the other part, restrengthening the diminished function of the part having the focus of disease. But if the pathological change is very great and the number of necrotic cells is very large, the irritation may become too strong and the normal part may be drawn in into the focus of the disease. Thus far I have tried to give

my explanation of the respiratory movement, and stated that although the respiratory movements are regulated by the action of the central nervous system, they are also enabled to perform their function by the irritation due to the direct action of the components of the dead cells of its own body which have entered the blood circulation upon the epithelial cells of the lung. If therefore, the components of cells are absorbed and enter the blood circulation in the living body, (it may be either the cells are dead and absorbed in the body, or they are parenterally injected in experiment), first they act upon the homologous cells, and quicken their physiological function and should the action be too strong, it causes the necrosis of the cells. *I call this action, the direct action of the components of cells parenterally injected, and would distinguish it from the action of organ toxin immunologically produced. And such direct action is what is always going on in the living body, and has a great significance for its physiological functions.* Only I have magnified its actions and made its reaction conspicuous in my experiment in order to make it apparent to our eyes. Turning to the application of such direct action, I deem that if it is properly utilized, it may give us the means to quicken the activity of tissue and organs whose function have diminished on account of various reasons. A treatment of anaemia is an instance. But I may take up this subject some other time.

The procedure of the formation of organ toxin.

If the homologous or heterologous cells are parenterally injected into the living body, the cytotoxin will be formed just as various kinds of immune bodies are produced by the injection of bacillus. (Ref. formula). If the cell *a* is parenterally injected into the animal *A*, after a certain period of incubation (from one to more than two weeks), a toxin is created in the body of *A*. When this toxin circulates in the various organs together with the blood, and comes upon the cell *a* in the body of *A*, the former acts on the latter, and if the reaction is very strong, the necrosis of the cell may follow. We can imagine the same phenomenon is going on in a living body by the action of the components of the cells that are dead and absorbed. Only the number of dead cells are not so many and the immune bodies thereby produced are not so much as that it is insufficient for as to able to prove their presence. But when the pathological change occurs in an organ, the number of dead cells becomes larger, and the above described direct action becomes conspicuous. And it has already been proved that these components become antigen forming various kinds of immune body and organ toxin. In a word, the formation of organ toxin has been investigated by many old scholars. More recently, many works concerning this subject have been made in our country under the supervision of *Mita*. The most celebrated is the research by *Nakamura* concerning the genesis of sympathetic ophthalmia. I and my co-workers have also made various experiments along this line. The experiments were directed to the investigation of the formation of

gastric ulcer, duodenal ulcer, inflammation or necrosis of kidneys, liver, testicles and lymph glands. Let me take an instance from the experiments on the formation of the gastric ulcer and try to explain it. Gastric ulcer is of course formed by various causes. But I shall discuss this subject from the point of view of the formation of organ toxin. The first man who directed his investigation to this subject is *Bolton.* Once a week, I injected the emulsion of the cells of the mucous membrane of the stomach of a rabbit into the peritaneal cavity of an other normal rabbit. After four or five injections, I could produce a serum which is virulent to the mucous membrane of that rabbit. When this serum was injected into the peritoneal cavity or the blood vessel or stomach wall of an othet normal rabbit, the ulcer was formed in the site of injection. *When it was injected into the stomach wall of the immunized rabbit itself, there also ulceration followed. These facts, which were first discovered by me, should have great significance for theorising on the genesis, progress and treatment of gastric ulcer.*

Kimura has made similar experiments concerning nephro-toxin. He injected nephro-toxin or the serum of the rabbit immunized with the kidney cells under the fibrous capsule of the kidney of one side of a normal rabbit. It has caused the remarkable necrosis of the parenchymatous cells in the kidney of the other side. This action having given too strong an irritation, caused the above-described change in the kidney of the other side There are many other experimental instances of a similar kind, but needless is to dercribe.

Whether or not the action of organ toxin upon the homologous cells is a cytolytic phenomenon, or whether or not, for instance, nephro-toxin dissolve the kidney cell, is the point concerning which we cannot make a hasty assertion. But I can prove the cytolytic phenomenon from the results of the examination of the action of gastro-toxin upon the cells of the mucous membrane of the stomach, the action of hepa-toxin upon the liver cells, the action of nephro-toxin upon the cells of the kidney in the test tube. But in order to determine whether or not these toxins are something like haemolysin, such investigation as the complement fixation test would be very much to the point. The question whether or not they are an antigen specific could be settled by the fact that gastro-toxin, hepato-toxin, nephro-toxin are easily absorbed respectively by the cells of the gastric mucous membrane, the cells of the liver, and the cells of the kidney.

It is usual that the cytotoxin *a* formed in the animal A is injected into the same kind of animal as *A*, it shows the reaction to the cell *a*. But sometimes no reaction appears. (Ref. formula). It is interesting to notice the appearance of reactions following the injection of the immune serum into the peritoneal cavity or the blood vessel of an other normal animal. For instance, when pneumo-toxin is injected into the auricular vein of the rabbit, a remarkable pathological change occurs in the lung, and when intraperitoneal injections of gastro-toxin, hepato-toxin, and nephro-toxin are made, the pathological changes respectively in the mucous membrane of the stomach wall, the liver and the kidney are most severe. This

shows the difference of organotropismus. Again when a serum of this kind is injected into a part of the respondent organ, the parenchymatous necrosis results as stated before. The formation of gastric ulcer and duodenal ulcer in our experiments are examples. The parenchymatous necrosis of the kidney produced by the injection of nephro-toxin into the fibrous coat of the kidney, the similar appearance produced by the injection of hepa-toxin under the cortical fibrous capsule of the liver, the remarkable change in the same side of the lungs caused by the injection of pneumo-toxin into the pleural cavity are other instances. This is the result of the concentrated mass reaction in one place. For the same reason, when the serum taken from an immunized animal is injected into a certain part of an organ of the same animal, and thereby a strange condition is introduced to the tissue, then the serum produces a mass action and the necrosis of parenchymatous cells occurs. What is called the auto-toxin of the digestive tract, kidney, liver is proved by it. It has indeed a great significance for the progress of the diseases of the said organs. When cell *a* is parenterally injected into an animal, it does not always show the reaction to the cell *a* in the body of that animal. In other words, there are cases in which direct action does not appear. Again the inoculation of cell *a* does not necessarily form the cytotoxin *a*. But it is not clear that the animal which does not undergo the direct action always lacks the the formation of cytotoxin. If we introduce already formed toxin or serum into the animals which lack the formation of cytotoxin, there is no reaction. Such an animal is to be regarded as refractory having a specific constitution.

The explanation of various forms of diseases by so-called direct action of the components of cells and action of organ toxin.

It is important and interesting to explain various forms of diseases from the genesis of the direct action of tne components of cells and the action of organ toxin. I shall describe one or two experimental instances. I may take the cases of nephritis due to mercurial toxining or nephritis due to the toxin of diphtherial bacillus toxin which acting directly on the cells of the kidney causes degeneration, necrosis and produces the inflammation of the kidney. A portion of the component of the parenchymatous cells necrosed by this change is eliminated with the urine and the other portion which is absorbed and enters the blood circulation acts directly upon the epithelial cells of the normal part, irritating the organ and quickening its function. The weakened functioning of the diseased part is restrengthened by this action. But if the direct action is too strong, the degeneration and necrosis of normal cells might result. If such direct action should continue for sometime, the nephro-toxin will be formed in a course of time, which circulation in the blood vessel will have nearly the same action on the normal part. These two actions working together will unite in equalizing and generalizing the appearance of nephritis. When there is orchitis in one side (for instance tubercular or

gonorrheal), the testicle of the other side becomes atrophic. This change occurs because the component of the dead parenchymatous cells of the diseased side directly acting on the cells of other side, induces the pathological change there, and this ultimately leads to the formation of organ toxin, which at first irritates and quickens the function, but later, on account of too great an irritation or going beyond the threshold of irritation causes the degeneration and necrosis of the cells of normal side and finally atrophy of the organ concerned. I believe that the fact that the hypertrophy of the testicle of the normal side will always appear after removing the diseased one is the proof of the truth I have been endeavoring to establish.

Summary.

When the component of dead cells in the living body is absorbed and enters the blood circulation, it acts directly upon the homologous cells and acclerates the formation of organ toxin which has also the action upon the homologous cells. Such a process is going on physiologically all the time. Because of this process, the physiological function of the cell receives an irritation. This irritation, co-operating with the regulative action of the nervous system enables the living body to perform the vital function in a good order. But if the irritation is too strong, or it goes beyond the physiological threshold of irritation, then degeneration and necrosis result. In other words, when the necrosis of cells is abnormally violent on account of various causes, the reaction becomes pathologically great. This point of view seems to teach us that diseases induced by various causes are more equally distributed over the whole of an organ by the direct action of the resorbed component of cells or the action of organ toxin, and display their typical forms. There have been many investigations concerning the organ toxin. But none has directed their attention to the *direct action* (specific term designated by me) of the absorbed component of cells upon the homologous cells. This has led me to explain what an important significance this direct action has on the life (physiological or pathological) of the living body.

An Experimental Study of the Cells of the Mucous Membrane of the Digestive Tract as Toxin or Irritant, and its Relation to the Formation of Peptic Ulcer.

By

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When the cells of the mucous membrane of the digestive tract are parenterally instilled into an animal, what action will it produce? And what action will the so produced cyto-toxin have on the cells of the mucous membrane of the digestive tract? To answer these questions, *Miyagawa* has been making an experimental study for years, and has already published some of his views on the subject. (The Journal of Path. and Bac. Vol. XXIII, 1920.) Since then, he has been carrying on his investigation, making experiments with large number of conclusions, and now with the confidence that he has reached a definite result, the following report is published.

Contents.

1. The action of rabbit v. rabbit immune serum immunized by the cells of the gastric mucous membrane of the normal rabbit. (The Formation of iso-gastro toxin and the production of ulcer.)

2. The microscopic appearance of the gastric mucous membranous tissue in the case of negative ulceration.

3. The cation of rabbit v. rabbit immune serum immunized by the cells of the normal gastric mocous membrane on the rabbit's own stomach, (the formation of autogastrotoxin, the formation of ulcer), and its significance in the formation of chronic gastric ulcer.

4. The formation of entero-toxin.

5. The action of rabbit v. rabbit immune serum immunized by the cells

of the normal gastric mucous membrane on the stomach of the guinea pig and the dog.

6. The cation of the immune serum of the immunized animal by the liver or the cells of the intestinal mucous membrane on the cells of the gastric mucous membrane.

I. The action of rabbit v. rabbit immune serum immunized by the cells of the mucous membrane of the normal digestive tract on the stomach of other normal rabbit. (The formation of iso-gastrotoxin and the production of the gastric ulcer.)

In the first report, *Miyagawa* published his view concerning iso-gastrotoxin. He was able to cause a remarkable ulceration in five rabbits by injecting the rabbit v. rabbit immune serum between the serous membrane and the muscular layer of the stomach wall of six normal ones. In this experiment, he injected twelve different kinds of immune serum into the same part of twenty-one normal rabbits, and caused the formation of remarkable ulcer in the stomach wall, especially in the mucous membrane of eight animals. Only one animal ended in the result $\pm$. To summarize, it is beyond doubt that the serum of a rabbit which has been immunized with the cells of the gastric membrane of the normal rabbit, after the elapse of a given time, has a cytolytic action on the stomach wall, especially on the cells of the mucous membrane of another normal rabbit, and causes the formation of ulcer in it. In other words, the cells of the gastric mucous membrane, when used as antigen, would from what is called "isotoxin." Let us describe a few instances.

(1) The rabbit No. 14 (Table I) the immune serum used was M5, VIII, 5 c.c. The cells of the mucous membrane of the stomach of a normal rabbit were injected into the peritaneal cavity of the rabbit M5 once a week. When a week elapsed after the eighth and last inoculation, the serum was collected and injected under the serous membrane of another normal rabbit. The injected animal became gradually inactive, followed by the decrease of appetite and emaciation. After a week, it was killed and dissected. The scar of the abdominal wall heald up well and there was no inflammation in the peritoneal cavity.

The stomach. The contents of the stomach were reduced to half the usual amount and had intermingled with a large amount of blood. The presence of mucous in them was noticed and the symptom of gastric catarrh was remarkable. So far as the serous membrane is concerned, there was no change in the site of the serum injection. But in its inner side, there were three haemorrhagic ulcers, the largest of which was of the size of a thumb. To the surface of the large ulcer, a clot was attached and hard to remove. The haemorrhage reached the lower layer of the mucous membrane and extended to a comparatively wide tract. The surroundings of the ulcer were somewhat hyperaemic, and there was a thickening of the mucous membrane. According to the microscopic examination.

mainly on account of hypertrophy of tissue, there were very few infiltrations and oedems. There was swelling in the surroundings, but the wall of the ulcer did not sink into sinus form. There were many parts in which the gradual healing toward normal mucous membrane was noticed. There was only one place which sunk into sinus form.

The second ulcer was quite adjacent to the first. It was of the size of a lens; its edge was infiltrated and thickened; its base reached the deeper muscular layer. The third ulcer was very small and appeared like that of haemorrhagic erosion. In other words, the ulcers showed the symptom of an acute type with hardly any tendence towards healing. Corresponding to the site of the injection of immune serum, there was ulcer formation. There is no room for doubting that the cause of this formation of ulcer was due to the action of the serum. Some might say, that such manipulation on the wall of stomach might mechanically induce nutritive deficiency, and concequently lead to ulceration. But this would be entirely mistaken idea. In many experiments made by ourself and by *Bolton*, there has been no change when the serum of the normal rabbit was injected under the serous membrane of the stomach wall of a rabbit. We can not tell beforehand what effect there will be if the injection is made too deep into the muscular coat, and part of the mucous membrane be injured. But the injection of serum under the serous memorane is by no means a difficult manipulation. The risk of doing a harm to the muscular coat by such injection is very little. But less will be the chance of doing injury to the mucous membrane. The formation of ulcer by immune serum is probably by the following process. The serum injected under the serous membrane, being absorbed by the lymph vessels, and a part of it by the blood vessels, reaches the mucous membrane where the cytolytic action by which the necrosis of cells is caused; then the cells are digested by incoming digestive fluid, and ulcer is formed. The serous membrane and the adjacent outer muscular layer may receive some mechanical injury, but this will very soon heal. The injected serum does not possess the specific immune body for the muscular cells, and the cells of the serous membrane. Hence the primary cytolytic action does not occur in these parts. But if an ulcer is formed on the surface of the mucous membrane, the action of digestive fluid may gradually deepen and finally reach the muscular coat; in such cases, this part may be digested, even causing punching out of the ulcer. This phenomenon was well shown especially in the experiments of *Bolton* and those of *Miyagawa* directed towards the investigation of heterogastrotoxin.

On the surface of the ulcer a remarkable haemorrhage was often noticed. Sometimes a ruptured haemorrhagic blood vessel was seen at the base of the ulcer. In the instance I am describing, this was recognized in the microscopic specimen. Whether this haemorrhage is to be regarded as caused simply by the necrosis of the cells of mucous membrane, and the consequent exposure of the blood vessel by the action of the digestive fluid, and the erosion of the vessel by

the digestive fluid needs further consideration. It may be questioned whether in the manipulation of isolating the cells of mucous membrane of one animal and immunising another animal with it, there may mingle with it some tissue of the blood vessels especially the endothelial cells acting as antigen, and whether the immune serum may contain this endothelial cytolysin. If that is case, one of the causes of the erosion of the blood vessels on the surface of ulcer must be placed there. The determination of this case can be made only after further research. The possibility only is mentioned here.

We shall briefly describe the microscopic appearance of the surface of the ulcer and of other organs?

The section specimen of ulcerated part.

The base of the ulcer reaches the muscular coat, a part of whose superlayer is digested by the action of digestive fluid and the other parts show a remarkable change. In the stomach muscles of the middle layer, various degenerative processes are noticed. Bur in the muscular coat nothing like cell infiltration is seen. The base which covers the ulcer is extremely thin, but the surrounding wall is very thick; and the part which adjoins the ulcer partly forms a sinus. In the ulcer, a large amount of necrotic tissue detritus and the blood corpuscles brought about by haemorrhage in one part, and various infiltrated cells in another part are discovered. On the surface of the surrounding mucous membrane there is very little inflammation. But there is a remarkable thickening and hypertrophy of the mucous membrane. The gland tubes are slender and narrow. The epithetial cells are small and short of granules. Moreover the distinction between the parietal and the principal cells is obscure. In spite of such hypertrophy, the part adjoining the ulcer makes a tongue-shaped protrusion. From the lower layer of the mucous membrane, the ulcer is intercepted by the necrosed patch, partly forming a sinus, partly passing gradually to the normal part. No tendency of healing is recognized.

The tissue of the mucous membrane of the stomach, excepting the ulcerated part.

Macroscopically, there is a catarrhal change of fairly high grade. The contents of the stomach are intermingled with a comparatively large amount of mucus. There is no haemorrhage and necrotic patch that could be macroscopically detected. In the nucleus of the pyloric gland cells, pyknosis or the vacuolar formation, and the decrease of chromatin are often noticed. When the pathological change is very great with the degeneration of protoplasm, very often the nucleus is lost. Even in the physiological and normal condition, the number of granules contained in protoplasm may differ according to the nature of its cells, but in the degenerated cells, there is vacuolar formation, and the distribution of

granules is very irregular, many of them sometimes aggregating in one place. Such cells when stained with sudan III. are discovered to contain much lypoid, much more than in physiologically seen minute granules. Sometimes such degenerated cells are seen in a train in a single gland. Sometimes a few such degenerated cells are mixed in a comparatively healthy gland. In the area of the phyloric gland, there is not much hyperaemia or haemorrhage. Only in the trasitional part of fundus and pyloric gland somewhat strong hyperaemia and microscopic haemorrhage are sometimes noticed. In the distribution area of the fundus gland and its principal cells, the state is very much the same as that of the phyloric gland just described. Here as in the case of the phylosic gland, the main part does not show any pathological sign. The only remarkable change is that of parietal cells. Here degenerated cells are intermingled with normal ones, but the former are fewer than the latter. The forms of degenerated cells are various; some long and spindle shaped, some exceedingly enlarged, some nearly triangular, some small and some spherical. Their positions in the gland tissue are very irregular; they are not always found on the outer sides; sometimes they look discolated out most noticeably toward the outer side, or toward the inner cavity of the gland: sometimes hard pressed by the normal principal cells, the parietal cells are usually found in the largest number at the neck of gland. But in some cases, they are mostly found in the duct or the outer layer of the gland, and only a few on the physiological part. It is not clear whether the cells physiologically located in the neck of the gland are degenerated and lost, leaving only those which are placed near the outer layer and which have stronger resistance, or the cells in the neck had been pushed out to the outer surface. The protoplasm of degenerated parietal cells is very unequal in staining. Of one cell, while a part is deeply, the other part only faintly stained and there is vacuolar formation in it. The arrangement of the granules in this case is entirely different from that of the physiological, and the cells contain much lypoid. The nucleus of degenerated cells is mostly in the state of pycnosis and is deeply stained. Its location is not fixed, on one side, or at one end, or in the middle of the cell. A vacuolar formation may also be found in it. These are the general change observed, and with regard to the causes of these changes, we state the following considerations.

(1) The injected serum may act on other normal parts by means of circulation.

(2) The formation of ulcer may cause a catarrh of the mucous membrane of the stomach, and give rise to these changes.

(3) A component of the cells of the mucous membrane of stomach, necrosed by ulceration may be absorbed and act directly on the other normal part of the mucous membrane of the stomach.

These actions taken together may account for the histological change of the normal part. In the muscular coat and the serous membrane no change is recognized.

The intestines.

The state is nearly the same in the duodenum, the small intestine and the large intestine. Although, in each of them, the part which does not show generally recognizable macroscopic change the greater part, there are slight hyperaemia and haemorrhage in some part. Generally, the greater part has no microscopic change. But in some parts, there is a slight change in the cells of the gland. In the cells of Brunner's gland and Lieberkühn's gland, the nucleus showed pycnosis or formed vacuole, and their location is very onesided. In the case of strong pathological change, the cells have no nucleus, and show very indistinct form. Various kinds of degenerative process are recognizable in the protoplasm. But such protoplasms are not no numerous. So far as fundamental change is concerned, there is very slight degenerative process in the essential cells.

The kidneys.

Generally there is slight hyperaemia, especially in the subcortical layer. There is no macroscopically recognizable haemorrhage. Microscopically, hyperaemia is recognized in the small bloood vessels along the uriniferous tubules and glomerulus. In some part, there is a haemorrhage from the small blood vessel, and the blood corpuscles are seen effused in the interstitial tissue or uriniferous tubules. But generally such a change is very slight, and there are not many haemorrhagic patches. Glomerulus is somewhat hyperaemic and has many round cells. There is no oedem. Its epithelial cells have some swelling, and their physiological forms are sometimes deformed. But the greater part has no noticeable change, In the epithelial cells of tortous tubules, we find one part which has undergone no change and a part which shows various degenerative processes. That part which does not show any change occupies the greater area. In that which shows the degenerative process, we find a swelling of the epithelial cells, very irregular formation, fatty generation and finally deformation of the cells. The change of the nucleus generally keeps place with it, showing in some cases, pycnosis, karyorhexis, vacuolar formation, a remarkable decrease of chromatin and finally the disappearance of nucleus. Sometimes the epithelial cells slough off into uriniferous tubules, but such cases are not very numerous. In uriniferous tubules there are found various kinds of casts. The hyaline casts are most numerous, but they are not composed of epithelial cells or of blood corpuscles. In many hyaline casts, various kinds of cells and granules are intermingled. In the straight collecting tube, the state is about the same, and the change is very slight. The change is especially noticeable in the epithelial cells of the descending limb of Henle's loop, but very little in the ascending limb. Generally, the change is great in the uriniferous tube around the glomerulus. By fat staining, the most intense change is noticeable in this part. The staining property is however not equal, the change being very great in several uriniferous tubules o one part, but not so striking in other parts. The same change may be noticed

in the case of haematoxylin eosin staining. The change in the interstitial tissue is like that of other part; slight hyperaemia and sometimes microscopically recognizable capillary bleeding. There is no large haemorrhagic patch, neither is there any infiltration of cells, in short, slight parenchymatous nephritis is noticed. When cells of any kind or immune serum are parenterally instilled into the living body, the kidneys generally act as a filter and excrete them outside. It is easy, therefore, to see that this organ is subject to degenerative change by some irritation. But the change is very slight compared to that which is caused by the parenteral injection of parenchymatous emulsion of the kidney or nephrotoxin.

The liver.

Macroscopically no change is recognizable. Microscopically following slight change in the liver cell trabecula is to be observed. In the cells near the central vein, there are vacuoles of various sizes, and many fatty granules; their glycogen content is much less than in the peripheral part of the liver lobules. But there are no cells in which nucleus has disappeared and which are in the state of necrosis. The degenerative process of this kind seems te have been caused by the action of a certain toxin coming from the central vein. It is essentially the same as the change which is caused by the parenteral injection of liver cells into a living body, only in the slighter degree. There is a slight congestion in the capillary blood vessels of the liver cell trabecula. Accordingly dilatation and hyperaemia of the central vein occurs. But there is no hyperaemia in the blood vessels of the liver lobules. In the bile duct, there is no congestion of biliary pigment.

The lungs.

The greater part has no macroscopic change. But there is a slight atelectasis or collapse of the lung bronchioles in a small limited part. Infiltration of various kinds of cells in the air vesicles occurs, and a hypertrophy of the connective tissue which compresses the air vesicles to such an extent that their expansion is sometimes rendered impossible. Slight oedem is recognized in the air vesicles wall. The cells found in the largest number in the air vesicle wall are the cells of connective tissue and the round cells. Pseudoeosinophilic cells, large mononucleolated cells, and lymphocytes are sometimes recognized. A few diapedesed red blood corpuscles are often found in this part. When atelectasis becomes stronger and macroscopically recognizable, the change in this part becomes stronger, and many basophilic cells, mononuclear cells and pseudoeosinophilic cells are found there. Whether all these changes are due to the action of gastro-toxin is a question. The part which shows such change is comparatively small, and the change itself cannot be compared to the change caused by the injection of the parenchyma of the lungs.

The suprarenal body.

In the study of the general organ toxins, such as gastro-toxin or nephrotoxin, this organ shows a very interesting side phenomenon. It is nearly always

attended with some parenchymatous change, although the extent of the change may not be the same in different cases. What we have now is an instance of a very slight change. But when the change is violent, macroscopically remarkable haemerrhage and necrotic patches are discernible. It might be interesting to give in this connection some description of it. The macroscopically discernible hyperamia or slight haemorrhage, and sometimes even a remarkable haemorrhage, is noticed in the cortical substance. In the medullar substance there is likewise hyperaemia and turbidity. On account of these changes, the suprarenal body often becomes swelled. The change of the parenchymatous cells is of a varying charcter. Of course, there are cases when no change occurs. In the case of degeneration, the lipoid content of the cells is not at all the same. Like that of the medullar substance, there is a general decrease of this latter in the cortical substance. Among the nuclei of the cells, there are those which manifest an almost normal physiological condition, and those which show a picnotic state and stain deeply. In their forms, some are nearly regular, some somewhat irregular, showing a slight pathological change. When the degenerative process to one side of the cell and sometimes zig-zag shaped. In a more violent case of change, staining becomes fainter, and with haemotoxylin-eosin stain, it takes the colors of these two substances and displays a reddish hue. In such a case, it is hard to discern the nucleous. A further step is the disappearance of the nucleus and a serious degenerative change with consequent necrosis of cells. Such degenerative processes as vacuolar formation and the irregular arrangement of granules are noticed in the protoplasm. Hyperaemia or haemorrhage can be recognized both macroscopically and microscopically in the medullar substance. In the parenchymatous cells of this part there are various changes. Even physiologically, some vacuolar formation may take place in this part. But in the present case, the change is greater, the nucleus contained in it becoming long, zig-zag or pycnotic, sometimes entirely disappearing. The above described change can be recognized more or less in any instances, but in this particular case the change is not great. But whence comes this change? It is the belief of the authors that the day is not distant when we shall be able to account for it. The change in the heart muscle, spleen, lymphatic gland and bone marrow is sometimes great, sometimes very little, sometimes non at all. But as their genetic change is similar to that caused by the injection of the cells of the mucous membrane of the digestive tract, its detailed account is omitted here.

(2) Rabbit No. 21 (Table I) MI, VI, 3 c.c. A rabbit was immunised by six injections of the cells of the mucous membrane of stomach. The serum was collected from it a week after the last injection, and 3 c.c. of it in each time was injected into two places in the stomach wall of the same rabbit. After a week, it was killed and dissected. Before killing, the rabbit was not lively, the desire for food decreased, and it was emaciated. The change in the stomach was as follows. In three places, ulcers of various sizes were formed. The largest one

was somewhat irregular in shape, twice as large as a pea in size. The base reached the deeper muscular layer. Its edge was covered with a dark necrotic patch. Its surroundings had become sunk somewhat in sinus form. No tendency towards healing was recognized. On the surface of the base a small clot was seen. The second ulcer was of the size of a pea, spindle shaped, and had infiltration of cells in its edge. Between these two ulcers a small bridge consisting of the mucous membrane at the brink of the necrosis, and connecting them, was seen. The third ulcer was of the size of a lens. Its base reached the deeper muscular layer, and its surroundings had become sunk in a sinus form. This ulcer was located a little apart from the other two. It is probable that it coincided with the site of the second serum injection.

Other organs. In the suprarenal body, the cortical substance was some what hyperaemic, the medullar substance had a slight oedem and turbidity. In the kidneys, there was generally strong hyperaemia, especially in the transitional part of the cortical and medullar substances. In lungs, liver, spleen and heart, there was hardly any macroscopically discernible change.

(3) Rabbit No. 18 (Table I) M2, VI, 3 c.c. A rabbit was immunised six times. 3 c.c. of its serum was injected into two places of its stomach wall. On the ninth day it was killed and dissected. Before killing, the animal had become emaciated and had little desire for food. It was comparatively lively. In two sites of injections, two haemorrhagic ulcers, the size of a thumb were formed. Around the ulcers, small haemorrhagic points were noticed. The change in the suprarenal body and the kidneys was similar to that of the former Rabbit No. 14.

In other instances, in the case of postitive result, similar ulcers were formed. The detailed description may be omitted here, and the general characteristics only have been shown in the table.

II. The microscopic appearance of the gastric mucous membrane in the case of negative ulceration.

We feel that it is also necessary to describe briefly the cases in which the formation of ulcer is negative, i.e., when it is not macroscopically discernible. In order to examine whether ulcer is formed or not by serum injection, the autopsy of the experimental animal is done usually after lapse of a week or so after the serum injection (unless the animal dies). A slight change on the surface of the mucous membrane of the stomach corresponding to the site of serum injection are occurred by mechanical injuries which is usually healed during that period, showing no traces. But it frequently happens that the microscopic examination of the suspected part will reveal the parenchymatous change of the cells of the glands, even in such pathological cases in which no macroscopic change is discerned. In such a case, the parietal cells show the difference in the property of staining, and so-called polychromatophilia. The size of the cells varies. Their arrangement in a single gland is unlike that of an ordinary case, the cells not taking the parietal

position over against the principal cells, and some of them being pushed out of the row of cells. A remarkable change is often noticed in the nucleus, which manifests picnosis and caryorrhexis. Sometimes the entire disappearance of the nucleus occurs. A similar change is recognised in the principal cells, the nucleus of which displays various kinds of degenerative processes: the cells sometimes entirely disappearing and sometimes forming vacuoles. The granules are very unequal. Even in one and the same cell, differences of staining indifferent parts may be noticed. Phenomena of this kind may be observable physiologically, but the change in this instance is greater. The arrangement of the cells in the gland is very irregular. There are parts in which no definite structure is observable on account of the necrosis of one or several cells of gland, the characteristic sign of the degenerative process. In such a part, there is very little resistance to the digestive fluid, and the consequent digestion by it, followed probably by the lack of material and formation of ulcer. The change however is slight in this case. Concomitant with this change the regeneration of the parenchymatous cells of the gland may be seen. Generally there is little change in the blood vessel. The diapedesis of red blood corpuscles is sometimes observed. There are cases in which comparatively strong haemorrhage occurs. Such a change may be the result of the change in the endothelial cells of the blood vessels. It is not impossible that a cytoxin produced by immunization may be present in the endothelial cells of the blood vessel and give rise to a specific action. The infiltration of the round cells in the interstitial tissue of mucous membrane and gland is not infrequent. But this kind of change is great in cases of remarkable ulceration and a strong catarrh, but slight when there is no ulceration. Thus it may be supposed that this change is not due to catarrhal inflammation.

The above-described change occur when the presence of the action of cytoxin can be microscopically proved. At the same time it is very difficult to determine the site of the serum injection, so we are not sure whether the section which we have taken as a specimen, may be out of the focus of the serum injection, and for that reason we were not able to find any appearance of pathological change. But I believe it is perfectly reasonable to think that the change in the cells caused by the injection of immune serum is of varying degrees, and there may be a very remarkable macroscopic change, a very slight just discernible microscopic change, and cases in which no change at all occurs.

III. The action of rabbit v. rabbit serum immunised by the cells of the normal gastric mucous membrane on the rabbit's own stomach, (the formation of autogastrotoxin, the formation of ulcer), and its significance for the formation of the chronic gastric ulcer.

Miyagawa stated in the first report that he had been able to cause a remarkable ulceration in producing a mass action by injecting the serum of a rabbit immunised by the cells of the normal gastric mucous membrane into its own

stomach wall. But in only one animal a positive result was obtained. Now in order to ascertain the result by testing many animals, the following experiment was made.

Under the serous membrane of the stomach wall of five immunised rabbits, was respectively injected an amount from 3 to 5 c.c. of their own immune serum. After from five to six days, they were killed and change of the injected parts were examined. Four of them showed typical ulcer and in one animal, there was no macroscopically recognizable ulcer. From this it may be concluded that the immune serum, when injected into the stomach wall, to the tissue of which it is placed in strange condition (what is called "gewebsfremd") produces a mass action which may causes the necrosis of cells and the formation of ulcer. This relation held true of his experiment on the liver and intestinal wall. The action of nephrotoxin observed by *Kimura* who made the experiment with him showed a similar effect. Whether or not such an action is actually present on the occasion of the genesis of the diseases in human or animal bodies is an unsettled question, but it is not at all inconceivable. Suppose the cells of the mucous membrane of the stomach are necrosed on account of various causes a portion falls into the intestinal tube, but the other portion (sometimes in an unusually large quantity) may be absorbed (it is not difficult to imagine that the absorption in this instance may be different from the physiological absorption); and as the action of the absorbed cells is entirely the same as that of the cells of the mucous membrane of the stomach parenterally infused, the component of the cells may have direct action on the cells of the same kind and act as an antigen producing a specific cytotoxin. Cytotoxin, so produced should have a specific action on the cells of the stomach wall. If in addition to this action, there were a second modus, it is very easy to imagine the production of a mass action. We may conceive the second modus in various ways. But even the first modus alone, namely, the necrosis of cells is sufficient to account for this pathological change. We do not insist that any ulcer in the stomach is caused by a specific action of cytotoxin. But it seems to us that none of the various modi, which have been enumerated up to this day, as the cause of an acute or chronic gastric ulcer satisfactorily explains the formation of the chronic round ulcer. We believe that when for some reason or other, the necrosis of cells takes place and the components of cells are absorbed, they act directly on the cells, and accelerate the production of cytotoxin which act again on the cells, themselves, augementing the destructive action of the first cause. Therefore we believe that the direct action of cell component resorbed upon living ones and cytotoxin thus produced should be counted as one of the modi which helps the formation, and especially hinders the healing of gastric ulcer.

It is our belief that from the specific action of autoserum the following statement can be made. When an ulcer is produced in a part of the stomach wall by various causes, and a portion of the cells is necrosed and their components are absorbed, there will be a production of a special cytotoxin; this cytotoxin

would have a toxic action not only on the cells of the stomach wall of an other normal body, but on the cells of the other part of its own stomach wall, acting as a cause which renders the healing of ulcer very difficult. In other words, it is not unreasonable to count the action of cytotoxin as a cause of the formation of the chronic ulcer.

Let us describe the nature of the ulcer by stating the courses in a few experiments.

(1) Rabbit No. 5 (Table I). For the immune serum, autoserum VII 5 c.c. was used. Once a week, seven injections of the cells of the stomach wall of a normal rabbit were made into the peritoneal cavity of the rabbit. After a week from the last injection, the serum was taken from and applied to the same rabbit. The laparotomy was made and 5 c.c. of the serum was injected between the serous membrane and muscular coat. There was a bulla in the site of injection, but no haemorrhage. Very soon it was absorbed. When the injection is made skilfully, no injury will be done to the stomach wall. The rabbit was emaciated and not lively, and its appetite decreased. On the sixth day of serum injection, it was killed and dissected. There was no suppurative inflammation in the abdominal wall and the peritoneal cavity. The stomach was soft and its content greatly reduced. The site of the serum injection was approximately on the transitional part of the gastric body and the gastric fundus. But apparently no change was externally noticed; only a bleeding from the small blood vessel at the base of the ulcer, and a clot were seen. The contents of the stomach were intermingled with mucus and altered black blood pigment. In the site of the serum injection of the mucous membrane there was an ulcer of the size of a pea. The wall of the ulcer was thick and showed an apparent adenomateous. The mouth of the ulcer was crater shaped and its edge sunk into sinus form. The base which reached the deeper layer of the muscular coat was very thin and was about to be punched out. On the surface of the base there was a small blood vessel from which there must have been bleeding and the ruptured part of the vessel was closed by a clot. In the mouth of the ulcer a transparent mucus was found. There was no inflammation in the surrounding of the ulcer. Neither was there hyperaemia nor haemorrhage. In a word, the ulcer had no acute reactive inflammation in its surrounding and showed no tendency to heal. These states resemble very much to the chronic round ulcer seen in the human body. (Ref. Plates). We shall next briefly describe the microscopic appearance of the ulcer and the appearances of other organs.

The ulcer.

The change in this part was similar to what we described concerning the action of isotoxin No. 14. Its base reaches the muscular coat. The muscle, deep as the middle layer, takes the degenerative process, and the outer wall of the ulcer is very thin. In the ulcer, there is a large quantity of necrosed tissue detritus and blood corpuscles. The part adjoining the mucous membrane is in sinus

form and shows no tendency to heal. The surface of the mucous membrane shows hypertrophy of cells and looks like the tissue of adenom. Generally there is little inflammation.

The gastric membrane in the unulcerated part.

The stomach generally show slight catarrh, and a large amount of mucus is intermingled with its contents. Besides that there is no macroscopically noticeable change. But microscopically there is a slight parenchymatous change. There is a far more serious change of gland cells in the gastric fundus than that described in No. 14. The most conspicuous change is found in the parietal cells. In the protoplasm, there are polychromatophilia, vacuolar formation, diminution of staining capacity, irregular arrangement of granules, and the greater fat content than in the physiological state. In the case of greater change, there is an entire disappearance of the nucleus. The size of degenerated cells is various. Their arrangement in a gland is not uniform. Some are located in the midst of the gland, some pushed outside. Many of them, instead of being found in the neck of the gland, seem pushed out toward the duct or superlayer. The various changes of uncleus, such as picnosis, vacuolar formation, diminution of staining capacity, their one-sided location on one side or at one end are observed. Their forms vary; zig-zag, spindle, small sphere, very irregular and shapeless, and finally the entire disappearance of nucleus itself. In the case of parenchymatous change, the nncleus is often unrecognizable. The above described changes pertain the parenchymatous change of the parietal cells. But similar changes of nucleus and protoplasm are seen in the principal cells. Sometimes there is a falling-off of the cells of the gland. The granules become larger, more numerous, and rather transparent. When the secretion ceases, they become smaller, less numerous, and remarkably blackened. Such a phenomenon is a common physiological occurrence. But the fundamental change in the form of the cells and the formation of much large vacuoles which takes place in this instance is not ordinarily seen. And on the surface of the mucous membrane, a remakably large quantity of detritual cells is found mixed with mucus. But such a change is not found everywhere. There are of course, many cells which undergo no such recognizable change. Even in one gland tubule, the cells which are degenerated are found mixed with those which are not degenerated. And generally, the change of gland cells is much slighter by comparison with the acute change caused by the parental injection of the emulsion of the cells of gastric mucous membrane. In the blood vessels of the mucous membrane and the submucous membrane there is often a slight hyperaemia. Concomitant with the change of parenchymatous cells, there is an increase of interstitious tissue, and sometimes slight infiltration of round cells. Generally, the mucous membrane is somewhat thin or atrophic. The haemorrhage in the area of the pyloric gland is slighter than in the gastric body and the gastric fundus. The mucous membrane is somewhat thin. Microscopically, the necrotic and detritual cells on the surface of the mucous membrane

are less than in the gastric fundus. There is not much mucus either. But so far as the parenchymatous cells are concerned, there is a relatively large number of degenerated cells, and as a consequence, not a few cells which contain a large quantity of fat, the changes in nucleus and protoplasm nearly correspond with those of principal cells of the gastric fundus. These are the vacuolar formation, the irregular location in cells, and sometimes the entire disappearance of nucleus. Such degenerated cells are present side by side with perfectly normal cells. But their number is not so great generally. There is almost no haemorrhage in the vessels of the mucous membrane and the submucous membrane. The above-described degenerative change in the normal part is essentially the same as that which is caused by the parenteral injection of the emulsion of the cells of gastric mucous membrane. The rabbit used for experiment had been inoculated seven times as it is hardly astonishing that such chages take place. Already with one inoculation a week, the animal was able to stand immunization. But now the mass action of autoserum accelerated a new formation of ulcer. As the result of it there was necrosis of cells, and a portion of their components was absorbed and acted directly on the cells. Again, the rabbit produced the immune body in its serum by immunization. So we must consider that this immune body will be carried to the normal part by circulation and act in a given way on that part. Moreover, on account of the above-mentioned actions, the mucous membrane of the stomach was affected with slight catarrh. This inflammation alone might produce a change to some extent in the parenchymatous cells. Hence it may be concluded that the synthesis of all these actions is the production of the changes which have been described.

The appearance of other organs.

Hardly any microscopic change is recognized in the intestines. There is no haemorrhage and no hyperaemia. We might even say, histologically there is no change. But when it is closely examined we can often recognize a slight degenerative process in the gland cells. The change, however, is very slight, only slightly greater in fact than seen in the physiological state. Such a change can be produced by the parenteral injection of the emulsions of the cells of the gastric mucous membrane, or by the formation of ulcer.

The kidney.

This organ is affected by the parenteral injection of the emulsion of almost any organ cells or organ toxin, and is a sort of filter through which the protein body or toxin, escapes. Macroscopic examination shows that there is somewhat strong hyperaemia under the cortical and medullary substances. There is no macroscopically recognizable haemorrhage and necrotic patch. In microscopic examination, the area of glomerulus is found to be in the severest haemorrhage. In some parts, there are a few bleeding spots and diapedesis of some blood corpuscles in the uriniferous tubules. There are slight oedems interspersed in the interstitial tissue and pressing on the uriniferous tubules. Generally, there is an increase of round cells besides haemorrhage in glomerulus. There is a similar

but somewhat greater change than that which is described in No. 14, in the tortuous tubules and Henle's loops. But in the interstitial tissue, there seems to be some increase of interstitious connective tissue besides blood corpuscles. It looks more like a chronic irritation than a by-phenomenon due to the acute gastric ulceration caused by autotoxin. This was no doubt produced during seven inoculations of the emulsion of the cells of gastric mucous membrane. This is quite different from the case in which the emulsion of the kidney war successively injected.

The liver.

Macroscopically there is very slight haemorrhage, but turbidity and swelling. Besides that, there is no other change. Microscopically, there is often somewhat strong hyperaemia along the liver cell trabecula ; but no haemorrhage. There is slight dilatation and hyperaemia in the central vein. In the liver cells around this part, degenerative processes, such as fatty metamorphosis, formation of vacuoles, changes in the nucleus are found. There is little glycogen content in such degenerated cells. This change is not recognized in the peripheral part of liver lobules. The change is exceedingly slight compared to that produced by injection of liver emulsion. There is no noticeable change in the blood vessels of liver lobules, bile duct, and the interstitial tissue of liver.

As the appearances of the suprarenal gland and the lungs are similar to those of No. 14, (above described) the repetition is saved. There is some change in the haemotopoietic, organs but its description is omitted here.

(2) Rabbit (No. 1.) (Table 1) M1 VII 5 c.c. The serum used was autoserum. This rabbit was injected with the cells of gastric mucous membrane of another normal rabbit. At the lapse of a week after the last injection, its serum was taken and injected under the serous membrane of its own stomach in the manner described before. The serum of the same rabbit had been injected into the stomach wall of a guinea-pig and formed an ulcer. On the sixth day of serum injection into the stomach wall the animal put under autopsy. In several parts of the peritoneal cavity, fibrinous inflammation was found, but no suppurative focus. Sedimentation of fibrins is doudtless due to the reactive inflammation caused by the injection of such solid substance as the emulsion of the cells of the gastric mucous membrane into the peritoneum cavity. The contents of the stomach are greatly reduced and watery, and intermingled with large quantity of mucus. There is no visible change in the site of serum injection. On the inner surface of stomach, there is an ulcer the size of the tip of the little finger. Around it there are oedematous swelling, hyperaemia, small haemorrhagic spots here and there, and a necrotic patch in the middle. In these haemorrhagic spots, there is papillary swelling and hypertrophy of tissues. The base of the ulcer reaches the outward muscular coat and generally there is a tendency to heal. In the other mucous membrane of stomach there is no remarkable macroscopic change. But there is generally a somewhat severe catarrh. Microscopically examined,

there is a relatively remarkable change in the gland cells. Various kinds of degenerative changes, atrophy or atresia of the gland cavity are noticed in the cells of gland, especially in the parietal cells. In the chronic catarrhal state, such a greater or less infiltration of round cells in the mucous and submucous membrane, and an increased secretion of mucus, is found. These changes must have been caused by various actions described before, e. g. the change of the cells of the stomach wall caused by the parenteral injection of the mucous membrane, or the direct action of the injected cells themselves, the action of cytotoxin produced by this immunization, the necrosis of cells by ulceration and the absorption of the so-decomposed components of cells which act directly on the cells of the mucous membrane of the other normal part, and the action of the catarrhal inflammation of the mucous membrane. But it is an admittedly difficult task to determine which is the main cause.

In other organs, the intestine, kidneys, suprarenal gland, liver, pancreas, lungs and heart, these is no remarkable change.

(3) Rabbit No. 9 (Table 1) M 3 VIII. 5 c.c. One week after the eighth immunization, 5 c.c. of its own serum was injected under the serous membrane of the stomach of the rabbit. The serum (an isotoxin) was one that produced an ulcer in the stomach wall of an other normal rabbit. We shall describe the change in the stomach wall only. There is no macroscopic change in the site of serum injection, But on the surface of the mucous membrane of the stomach there is a formation of a remarkable ulcer, the size of a pea. Its surrounding wall sinks into sinus form with little tendency to heal. Its base reaches the deeper layer of the muscular coat. The sites are atrophic and have some oedem. Microscopically, it shows an adenoid tumor. There is no inflammation. The mucous membrane in other parts is similar to that described in No. 1.

In other instances, the states are similar and the detailed description will be omitted. To sum up. That the parenteral injection of the cells of the mucous membrane of the stomach of a rabbit into another rabbit produces in its serum a substence which has the property of giving rise to toxic action on the cells of gastric mucous membrane, has been stated in the description of the action of isogastrotoxin. That this serum when brought to the condition alien to the tissue of the mucous membrane of stomach, that is, when it is taken out of the blood vessel and injected under the serous membrane of the stomach wall, is absorbed and reaches the mucous membrane, causing toxic action and the necrosis of cells and formation of ulcer, has also been stated. We also stated that whether such action actually occurs in the natural or pathological states of human being is an open question. But can we experimentally prove at any time this toxin formation or auto-toxining phenomena? We can hardly give the answer affirmatively. But we shall try to explain this point later.

Table I.

Injection of rabbit v. rabbit immune serum into the stomach wall of rabbit, guinea pig and dog (gastrotoxin).

Date	No.	Injected animal	Injected serum	Autopsy	Appearance	Result
2–7Nov. 1921	1	Rabbit (Its own immunised Stomach wall)	M_1 VII 5 c.c. Into stomach wall (Auto serum)	Six days after inoculation was killed with chloroform	Before described ulcer the size of tip of little finger in injected part tendency to heal	Positive Auto (+)
2-4 „ „	2	Guinea pig (Normal)	„ „ 2 c.c. Into stomach wall	Dead within 48 hours from inoculation immediate autopsy	Ulcer the size of tip of little finger in site of injection, hyperaemia in gastric body and gastric fundus	Positive Iso (+)
2–10 „ „	3	Rabbit (Normal)	„ „ 5 c.c. „ „ „	six days after inoculation was killed	no change in site of injection	Negative Iso (−)
3–10 „ „	4	Dog (Normal)	„ „ „ „ „ „	A week after inoculation was killed with morphine	„	Negative Iso (−)
2–7 „ „	5	Rabbit (Its own immunised stomach wall)	„ „ „ „ „ „	Relatively normal Six days after inoculation was killed	Ulcer size of a pea very thick wall ulcer reaches muscular coat	Positive Auto (+)
„ „ „	6	Rabbit (Normal)	M_2 VII 5 c.c. Into stomach wall	Six days after inoculation much enfeebled and dying was killed with chroloform	No chang in site of injection	Negative Iso (−)
2-6 „ „	7	Guinea pig (Normal)	„ „ 2 c.c. „ „ „	Relatively normal Five days after inoculation was killed	„	Negative Iso (−)
3–10 „ „	8	Dog (Normal)	„ „ „ „ „ „	Normal a week after inoculation was killed with morphine	„	Negative Iso (−)
3–12 „ „	9	Rabbit (Its own immunised stomach wall)	M_3 VIII 5 c.c. Into stomach wall	Emaciated and enfeebled Five days after inoculation was killed	Typical ulceration in site of injection	Positive Auto (+)
8–12 „ „	10	Rabbit (Normal)	„ „ „ Into stomach wall	„	Ulcer size of pea tending to heal	Positive Iso (+)
„ „ „	11	„ „	M_4 VIII 5 c.c. Into stomach wall	Five days after inoculation was killed no change in liveliness	No change	Negative Iso (−)

8–12 Nov. 1921	12	Rabbit (Normal)	M_4 VIII 5 c.c. Into stomach wall	Five days after inoculations was no change in liveliness	No change	Negative Iso (−)
22–28 „ „	13	Rabbit (Its immunised stomach wall)	M_5 VIII 5 c.c. Into its own stomach wall	A week after inoculation was killed	No macroscopic change	Negative Auto (−)
„ „ „	14	Rabbit (Normal)	„ „ „ „ „ „ „	Emaciated, not lively a week after inoculation was killed	Ulcer size of a thumb, ulcer size of lens by side of former	Positive Iso (+)
„ „ „	15	„ „	„ „ „ „ „ „	No change in liveliness a week after inoculation was killed	No change	Negative Iso (−)
7–15 Dec. 1921	16	„ „	M_{12} VI 3 c.c. Into stomach wall	Nine days after inoculation was killed	„	Negative Iso (−)
„ „ „	17	„ „	„ „ „ „ „ „	„	„	Negative Iso (−)
„ „ „	18	„ „	„ „ „ „ „	Emaciated, not lively nine days after inoculation was killed	Ulcer size of thumb	Positive Iso (+)
22–26 „ „	19	„ „	M_{17} VI 1.5 c.c. Into stomach wall	Five days after inoculation dead	No change in site of injection	Negative Iso (−)
21–28 „ „	20	Rabbit its own immunised stomach wall)	M_{16} VI 3 c.c. Autoserum	A week after inoculation was killed	Ulcer size of lens	Positive Auto (+)
„ „ „	21	Rabbit (Normal)	„ „ „ Stomach wall serum	„	Three ulcers in various sizes	Positive Iso (+)
10–11 Jan. 1922	22	„ „	M_{10} 3 c.c.	Dead within 24 hours from inoculation immediate autopsy	Egg sized hemorrhagic spots, several necrotic patches	Positive Iso (+)
9–13 „ „	23	„ „	M_9 3 c.c. Into stomach wall	Five days after inoculation was killed	Ulcers size of tip of little finger, size of lens in three places	Positive Iso (+)
„ „ „	24	„ „	„ „ „ „ „	„	Erosion of mucous membrane in site of injection	Dubious Iso (±)
9–17 „ „	25	„ „	„ „ „ „ „	Nine days after inoculation was killed	Remarkable formation of ulcer size of thumb	Positive Iso (+)
22–26 „ „	26	„ „	„ „ „ „ „	„	Ulcer size of pin head	Negative Iso (−)

17-20 Jan.	27	Rabbit (Normal)	M_{IB} 3 c.c.	Four days after inoculation was killed	No change	Negative Iso (−)
,, ,, ,,	28	,, ,,	M_{II} 3 c.c.	,,	,,	,,

IV. The formation of entero-toxin.

We tried the same manipulation to the cells of the mucous membrane of duodenum as we did to the cells of the mucous membrane of stomach wall. We injected with the immune serum thus obtained under the serous membrane of the duodenum of the same or other normal rabbit, and examined its action. When 2 c.c. of the immune serum was used, we were able to produce remarkable ulcers in two immunised rabbits, and prove the formation of auto-enterotoxin. Applying it to seven normal rabbits, we recognized the formation of typical ulcers in two of them. From the result of these experiments, we might say that the cells of the mucous membrane of duodenum have entirely the same property as the cells of the gastric mucous membrane from the point of view of immunology or of the formation of cytotoxin.

Let us briefly describe the instances shown in the table. A rabbit was immunised twice with the cells of the mucous membrane of duodenum. A week after the last inoculation, 2.0 c.c. of its serum was injected under the serous membrane of its own duodenum. A week later, it was killed and put under autopsy. The animal had lost liveliness and had no desire for food, and was emaciated. At the time of the autopsy, there was no abnormal inflammation, but deposition of fibrin in the peritoneal cavity. Externally there was no change in the wall of duodenum. In its inner side, there was an ulcer the size of the tip of the little finger. Its edge was thickened; its surface was sunken in the form of a sinus; its base reached the deeper layer of mascular coat. There was bleeding from the small blood vessel in the base, on account of which the inner surface of the ulcer was covered with a clot. This ulcer showed no tendency to heal. There was no reactive inflammation around the ulcer. It looked just like a chronic ulcer of duodenum. There was no change in the stomach and other part of intestine. In the suprarenal gland there was turbidity in its medullary substance, and hyperaemia in its cortical substance. Generally the kidneys were somewhat hyperaemic, especially in the lower layer of the cortical substance. These changes were noticed in other ulcerated animals (isotoxin), and in the immunised animals (autotoxin). The changes are probably due to the action of cytotoxin produced by the direct action of the component of the cells of the mucous membrane of duodenum which was absorbed and passed into circulation. The result of this expnriment entirely agrees with the report of *Murai*. So we shall not enter into the details.

Table II.

Enterotoxin.

Date	No	Injected animal	Injected serum	Autopsy	Appearance	Result
15-21 Dec. 1921	1	Rabbit (Normal)	D_{12} II 2 c.c. Duodenal wall	A week after inoculation was killed	No change	Negative Iso (−)
,,	2	Rabbit (Normal)	Immune serum	,,	No change	Negative Iso (−)
,,	3	Rabbit (Its own duodenal wall)	Its own serum 2 c.c. D_{12} II	,,	Ulcer the size of the tip of little finger in the site of inoculation, reached muscular coat	Positive Auto (+)
10-11 Jan.	4	Rabbit (Normal)	D_{11} V 2 c.c.	Died within 24 hours from inoculation. Immediate Autopsy	No change	Negative Iso (−)
9-13 Jan. 1922	5	Rabbit (Its own duodenal wall)	Its own serum 2 c.c. D_{11} V	Five days after inoculation was killed	Ulcer the size of a lens	Positive Auto (+)
,,	6	Rabbit (Normal)	D_{11} V 2 c.c.	,,	Ulcer the size of a lens; the surrounding wall, thickened	Positive Iso (+)
9-17 Jan. 1922	7	,,	,, 1 c.c.	,,	,,	Positive Iso (+)
17-20 Jan. 1922	8	,,	,, 2 c.c.	Four days after inoculation was killed	No change	Negative Iso (−)
,,	9	,,	D_1 VII 2 c.c.	,,	,,	Negative Iso (−)

V. The action of rabbit v. rabbit immune serum immunised with the cells of the mucous membrane of a normal stomach on the stomach of the guinea-pig and the dog.

A few years ago, *Miyagawa*, one of our co-workers had published a statement that rabbit v. rabbit serum immunised with the cells of gastric mucous membrane acts on the stomach of the guinea-pig. So to reexamine the appearance thus obtained, we have made many experiments on the following several animals and have obtained the same appearance.

Guinea-pig, (normal). (Rabbit M_1 serum VII 2 c.c.)

Having performed laparatomy on a guinea-pig, we injected 2 c.c. of rabbit v. rabbit immune serum under the serous membrane of its stomach wall. The serum had been immunised once a week with seven inoculations of the cell of the mucous

membrane of the normal rabbit, and taken after a week from the last inoculation. The guinea-pig showed the symptom of poisoning immediately after the serum injection, and recovered in nearly two hours. This symptom is almost invaluably seen. On the following day, the animal was again not lively, had no dasire for food, and died in about twenty four hours. It was immediately put under autopsy. In the abdominal wall and the abdominal cavity there was no suppurative process. There was a strong hyperaemia along the lesser curvature of the stomach. The same was true of the bordering area of the fundus and pyloric glands or isthmus. The serum injection was made at the anterior wall of the gastric body, near the transitional part of two kinds of glands. Dark haemorrhagic spots were seen in this part. It was difficult to determine whether it was the mechnical bleeding under the serous membrane caused by the injury of the small blood vessels received on the occasion of serum injection, or the action of immune serum. Probably the former may be the cause as their site was under the serous membrane. The contents of the stomach were greatly reduced, but the stomach expanded with gas. This is always seen in the case of remarkable ulceration in the stomach. Even in the rabbit's stomach which is nearly all the time filled with food, this fact may be noticed.

The formation of gastric ulcer. In the site of serum injection there was an ulcer the size of the tip of little finger. Its base was covered with dark altered blood pigment. There was necrotic tissue. Closely examined, it was found that in the surrounding of the ulcer, there were some oedems, infiltration of cells and consequent swelling. The wall of the ulcer did not sink into the sinus form. It gradually sloped down in a concave shape and passed to the central part. Really it showed the marks of typical acute ulcer. In other parts, there was no macroscopically noticeable pathological change in the mucous membrane. The hyperaemia seen in the lesser curvature and on the serous membrane must be the result of the mechanical injury done by taking hold of the stomach wall at the time of operation. Accordingly there were no changes whatever on the surface of the mucous membrane.

There was slight hyperaemia in the liver but no haemorrhage. There was oedematous swelling and a strong hyperaemia in the medullary substance of the suprarenal gland. In the cortical substances, there was remarkable hyperaemia. There was no noteworthy change in the kidneys, intestine, spleen, heart and lungs.

From these appearances, the following may be concluded. The serum obtained from the rabbit immunised with the mucous membrane of the stomach of other normal rabbit, has the cytolytic action on the stomach wall of a different kind of animal, viz. a guinea-pig, and is able to produce in it necrosis and the formation of an acute ulcer. The experiment made by *Miyagawa*, a few years ago in the University of London, England, in which five different kinds of rabbit v. rabbit immune sera were injected into sixteen guinea-pigs, and three formed

ulcers, two showed dubious results, and others showed no change. Thus when the rabbit v. rabbit immune serum is applied to the guinea-pig, the action is not so certain as on the stomach of the rabbit. But when a large number of test animals were experimented on, sometimes remarkable actions were noticed. (Ref. plates.)

Laparatomy having been performed, the rabbit v. rabbit immune serum was injected into the stomach walls of two dogs, and the changes were examined. In neither of the two, any reaction followed. But the experiment of this kind needs a large number of test animals, for a valid conclusion can be drawn only from the results obtained from experiments on many animals. We cannot infer that the rabbit v. rabbit immune serum does not act on the stomach of the dog from this experiment. It is the author's belief that the rabbit v. rabbit immune serum is able to form ulcer in the stomach of a dog, provided that a fairly large number of test animals are experimented on. I (*Miyagawa*) in my experiment in London University, found that the guinea-pig v. rabbit immune serum acts not only on the guinea-pig, but also on the stomach wall of the rabbit. In such manipulation we cannot expect always to get a positive result. So quoting what I published then: "The rabbit v. rabbit immune serum contains a specific gastrolytic factor to the guinea-pig's stomach cells, although the cytolytic power is uncertain and not constant owing to the individuality of the immunised rabbit and also to that of the guinea-pig used for serum injection," and, "The guinea-pig v. rabbit immune serum is able to act not only upon the guinea-pig's stomach but also upon the rabbit's stomach. It is, therefore, apparent that the guinea-pig's and rabbit's stomach cells have similar side chains." I believe this is a valid conclusion.

VI. The action of the immune serum of the animal immunised with the liver cells or the cells of the entestinal mucous membrane on the cells of the gastric mucous membrane.

It is clear from the experiment of *Miyagawa* and our experiment on the immune serum of the cells of the mucous membrane of the stomach and intestine, and the immune serum of liver, kidneys, lungs, testes and white blood corpuscles, that the serum obtained by the inoculation of the organ cells, acts upon the organ cells of the same kind. Is this action on the parenchymatous cells specific or not? This is an important question and must be settled by a careful investigation. In order to examine this point in the living body, the following experiment has been made.

Three kinds of entero-toxin were injected into the stomach wall of six rabbits and the examination as to ulceration was made. No reaction whatever was noticed in the site of injection. We believe this was because our experiment was made on too small a number of animals, and we could have formed ulcer in the stomach wall with entero-toxin, if we had used a larger number of teste animals. But from the result of our few experiments it is undoubtable that the

action of enterotoxin on the cells of the mucous membrane of the stomach wall is not so strong as that gastrotoxin.

We formed hepatoxin by injecting the liver cells emulsion into a rabbit and introduced the so-obtained strong serum into the stomach wall and examined the reaction. In the same way, we prepared three kinds of hepatoxin and injected them into the stomach wall of eight rabbits. In one animal there was no change in the outer layer of the stomach wall, but there was an extensive haemorrhagic patch, here and there necrosis of mucous membrane and ulcerous surfaces. In other animals, there was an ulcer having the nature described in the table and already tending to heal. There was no change in others. From this experiment it will be seen that hepatoxin has a similar action on the cells of the mucous membrane of the stomach wall as that of gastrotoxin, and causes the necrosis of cells which are digested and forms ulcer. The action of these heterologous organ cells may be something like the group reaction in bacterioimmunology. We call the action on the homologous cells the principal, this action is called the accessary reaction. If we apply complement fixation for the investigation of this phenomenon, the relation between them will be made clearer.

It is the author's hope that further research on this subject will enable him to state the relation of the principal and accessary reactions in more elucidating terms.

Let us conclude by mentioning the interesting result of our experiment. When gastrotoxin is injected into the abdominal cavity of an other normal animal, the part affected is mainly the cells of the gastric mucous membrane. The haemorrhagic and necrotic patches are produced and ulcers are formed in this part. It is clear therefore, that the so-formed toxin has a specific affinity to the homologous cells. (Ref. Plate). In the cases of hepatoxin and nephrotoxin, the same holds true. Is there no change at all in other organs? We might say there is more or less change in them also. But it is very slight and in many cases non at all. Whether we have to regard such a change as an accessary reaction or not is to be determined by further investigations.

Table III.

Local action of the immune serum of intestinal mucous membrane or of liver injected to stomach wall.

Date	No	Injected animal	Injected serum	Autopsy	Appearance	Result
8–12 Nov. 1921	1	Rabbit (Normal)	D_1 IV 5 c.c. Into stomrch wall	Five days after inoculation was killed	No change	Negative
,,	2	,,	,,	,,	,,	Negative
,,	3	,,	,,	,,	,,	Negative

22–27 Nov. 1921	4	Rabbit (Normal)	D_2 VI 5 c.c. Into stomrch wall	Six days after inoculation was killed	No change	Negative
22–23 „	5	„	D_3 VI 5 c.c.	Killed on the following day	„	Negative
22–28 „	6	„	„	A week after inoculation was killed	„	Negative
29 Nov.– 1 Dec.	7	„	L_1 VII 3 c.c. Into stomach wall	Dead on the third day. Immediate autopsy	No change in outer layer of stomach wall, extensive haemorrhagic focus in mucous membrane, here and there necrosis, ulcer	Positive
„	8	„	„	Three days after inoculation was killed	No change in stomach	Negative
29 Nov.– 3 Dec.	9	„	„	„	Small ulcer in stomach already tending to heal	?
30 Nov.– 3 Dec.	10	„	L_2 VIII 3.5 c.c. Into stomach wall	Four days after inoculation was killed	No change in stomach	Negative
„	11	„		„	„	Negative
1–2 Dec. 1921	12	Rabbit (Immunised with liver)	Its own serum 3.5 c.c. into Stomach wall (I^2)	Dead on the following day.	„	Negative
30 Nov.– 1 Dec. 1921	13	Rabbit (Normal)	L_3 VIII 3.5 c.c. into Stomach wall	„	„	Negative
„	14	„	„	„	„	Negative

VII. The tissue or organ cells as toxin or irritant (especially the cells of mucous membrane of digestive tract) and formation of organ toxin.

Cell (a)→Animal A
- → Cell (a) Reaction (−) → Formation of Cytotoxin (−)—*
- → Cell (a) Reaction (+) → Formation of Cytotoxin (+)—**

* → Cell (a) Reaction in its own body and body of other animal (−)
- → Cell (a) Reaction in other animal's body (−)

** → Cell (a) Reaction in animal A's body (+)
- → Cell (a) Reaction in other animal's body (+)

When the cell (a) is parenterally instilled into the animal A, it would act on the homologous cells, and wirh a certain period of incubation, it will form a cytotoxin in the body of the animal. But as there is a difference of strength in the reaction of the direct action produced by the injection of the cell (a), so there is a difference of strength in the cytotoxin thus produced. Owing to the individuality of the animal, there may be no formation of cytotoxin. Perhaps the antitoxin may be formed against the cytotoxin. For the animal which is extremely sensitive to a small quantity of injected organ cell gets its tolerance increased with the repetition of immunizing manipulation, or the increase of the number of injections just as in the case of bacterial immunization. We cannot regard it merely as the result of the animal becoming accustomed to it. Therefore when a certain amount of antitoxin is produced, thero may arise a defensive action. The appearance of an organ of an immunized animal i. e. the pathological change of an organ due to the direct action may be worst in the begining of immunization, and gradually disappear with the repetition of injections until the organ is restored to its normal state as in the case of the cells of mucous membrane of the stomach and the intestine. When an organ, (e. g. kidneys or liver), howerver, brings necrosis beyond a certain limit on its parenchymatous cells, its perfect regeneration becomes impossible. Sometimes there is a thickening of interstitial substances. Even in such a case there is of course, the regeneration of parenchymatous substance. But then the degenerative change of the parenchymatous substance and hypertrophy of interstitial substance is overwhelmingly strong compared to the weak regenerative process. As shown in above formula, there is no doubt that the formed cytotoxin has a definite action on the homologous, sometimes on the heterologous cells of the same boby. This is clear also from the appearance in our experiment of the cells of the mucous membrane of the digestive tract, liver, kidneys and lungs. But the appearance produced by the parenteral injection of thus produced cytotoxin into the animal of the same kind is various; the reaction being either positive or negative. In other words, there is a difference in susceptibility. For the same reason, the reaction produced by the injection of cell "a" into animals having a different susceptibility may differ in point of its strength, and in some cases, the result may be negative. Some explanation of the table showing the action of gastrotoxin may not be out of place. That the strength of the formation of cytotoxin varies according to the individuality of animals may be seen from those appearances. The serum M4 produced no macroscopically local change in two normal rabbits. The serum M5 which did not produce any macroscopically noticeable change in the rabbit's own stomach wall formed a typical ulcer in one of two normal rabbits experimented upon. The serum M3 formed ulcers both in the rabbit's own and the other stomach wall. The serum M9 had formed such strong cytotoxin that it caused ulcers in all four normal rabbits to which it was applied. Thus there is a great variation in the formation of cytotoxin according to the individuality of animals. There is

doubtless also the variation of strength of reaction produced by the injection of such cytotoxin into an other normal body. The variations may be the macroscopically recognizable remarkable ulceration, the slight microscopical change in the cells of gland, and no change whatever. Whence come these variation? It is very difficult to answer. All we can say at the present stage of knowledg is that they are due to the difference of susceptibility of each individual animal.

We shall express our opinion concerning the questions whether such ulceration caused by the action of above described auto-gastrotoxin or auto-enterotoxin actually happens at the moment of the natural pathological process.

There is no room for doubt that in the diseases of the digestive tract, the cells of the mucous membrane are necrosed, and a part of their components may fall off, and the other part may be absorbed in its abnormal state. And it is not difficult to imagine from the above-described experiments that these absorbed cell components would act as antigen in its own body and make cytotoxin. It is clear from our own experiment and our co-worker's results, and similar works by others that there will be formations respectively of gastrotoxin, hepatoxin and nephrotoxin together with some specific histological changes in some other normal part, when a portion of the stomach, or a bit of liver, or an end of a kidney is mechanically smashed. It is a matter of course, that the cytotoxin thus formed will extend its action to other normal part. And it can easily be imagined that there will arise functional disturbance in the organ in the individual in which such cytotoxin is formed. Moreover, if there were some idiosyncracies in the organ, (e. g. if in the stomach there were excess of hydrochloric acid, over excitement of tonus of vagus nerve, abnormality of the blood flow in gastric wall, disturbance of the discharge of the contents of stomach or some other cause which would produce necrosis of cells of the gastric mucous membrane, and continue to exist then the cytotoxin thus produced in its own body will all the more powerfully act on the locus minoris resistentiae in the stomach. Therefore we believe that the absorbed components of necrosed tissue and accelerate the formation of cytotoxin which would again act on the ulcer, rendering its healing a matter of great difficulty and efficiently acting as a cause of the chronic ulcer. We do not mean to say that the direct action of resorbed cell components and the formation of cytotoxin would explain the genesis of all acute and chronic ulcers in the digestive tract. But we believe it can be regarded as a modus of the formation of such ulcers and a cause of making them chronic.

Conclusions.

(1) We proved the formation of iso-gastrotoxin and iso-enterotoxin. By using them, we were able to produce ulcers in the digestive tract of other normal animals.

(2) We proved the formation of auto-gastrotoxin and auto-enterotoxin. By using

them we were able to form ulcer in the animal's own body.

(3) The formation of cytotoxin varies according to individuality. The susceptibility of other normal animals to the thus-formed cytotoxin also varies.

(4) We believe that the direct action of resorbed cell components of the digestive mucous membrane and gastrotoxin and enterotoxin are to be regarded each as a modus of the genesis of ulcers in the digestive tract. The direct action of resorbed cell components and the action of cytotoxin renders to recovery of ulcer produced by various other causes very difficult.

(5) Iso-gastrotoxin of the rabbit can act on the stomach wall of guinea-pig and form ulcer in it.

(6) When enterotoxin and hepatoxin acts on the stomach wall, often there are reactions, and the formation of ulcer may sometimes be seen. But such reactions are not so certain and constant as that of gastrotoxin on the stomach wall.

Explanation of Plates.

Fig. 1. Rabbit No 5. gastric ulcer produced by the action of auto-gastrotoxin.
Fig. 2. Rabbit's stomach. Two ulcers produced by the action of iso-gastrotoxin.
Fig. 3. Rabbit's stomach. Gastric ulcer formed by the action of auto-gastrotoxin.
Fig. 4. Guinea-pig's stomach. Many haemorrhagic erosions and superficial ulcers produced by the intraperitoneal injection with rabbit v. rabbit immune serum. (iso-gastrotoxin)
Fig. 5. Rabbit's stomach. Two ulcers formed by the action of iso-gastrotoxin.
Fig. 6. Rabbit's stomach. Many ulcers produced by the action of iso-gastrotoxin.
Fig. 7. Rabbit's duodenum. Duodenal ulcer formed by the action of auto-enterotoxin.
Fig. 8. Rabbit's stomach. Gastric ulcer produced by the action of auto-gastrotoxin.
Fig. 9. Rabbit No. 14. A remarkable ulcer formed by the action of iso-gastrotoxin.
Fig. 10. Rabbit's stomach. Gastric ulcer produced by the action of auto-gastrotoxin.
Fig. 11. Guinea-pig's stomach. The upper one shows a wide necrotic patch produced by the action of rabbit v. rabbit immune serum injected intraperitoneally. The lower one shows normal appearance of gastric mucous membrane of the guinea-pig.
Fig. 12. The section of the ulcer of rabbit No. 5.
Fig. 13. do. of rabbit No. 14.
Fig. 14. The section of an ulcer produced by auto-gastrotoxin.
Fig. 15. do. by iso-gastrotoxin.
Fig. 16. Hypertrophy of the surrounding tissue of the ulcer of rabbit No. 5.
Fig. 17. Haemorrhagic patches in the surrounding of the site of serum injection.

Fig. 1.



Fig. 2.



Fig. 3.

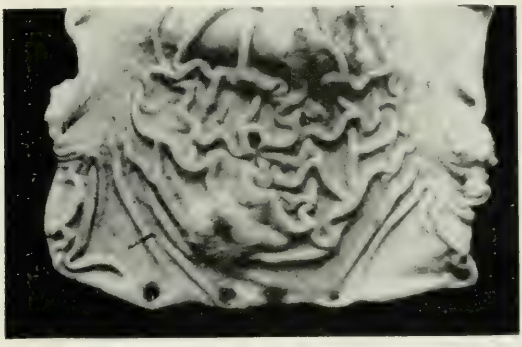


Fig. 4.



(Dr. Y. Miyagawa : An Experimental Study of the Cells of the Mucous Membrane of the Digestive Tract as Toxin or Irriant, etc.)

Fig. 9.

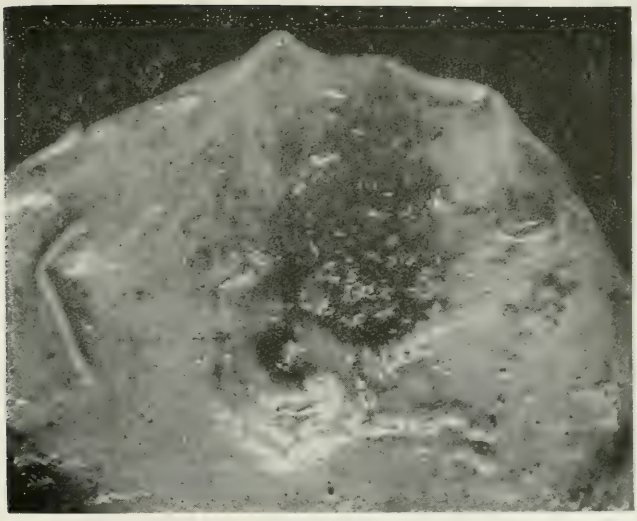


Fig. 10.

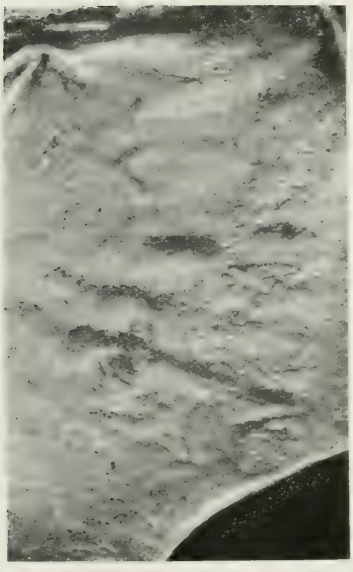
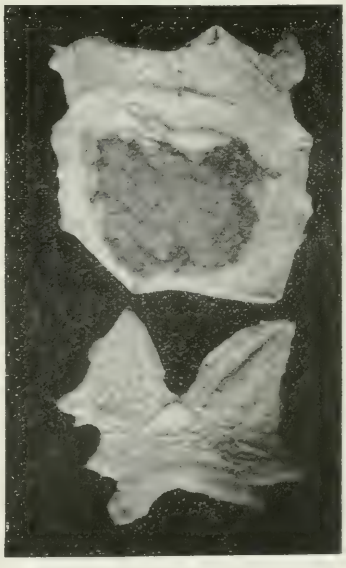


Fig. 11.



(Dr. Y. Miyagawa : An Experimental Study of the Cells of the Mucous Membrane of the Digestive Tract as Toxin or Irriant, etc.)

Fig. 12.

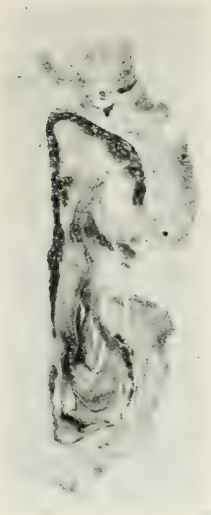


Fig. 13.

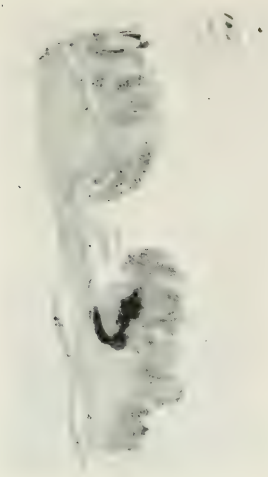


Fig. 14.

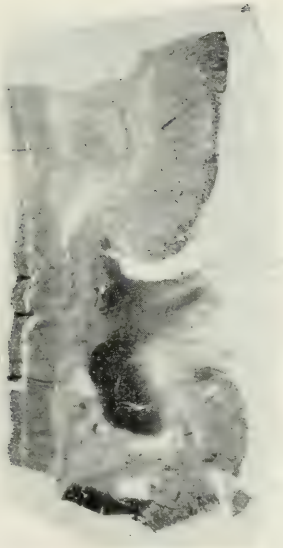
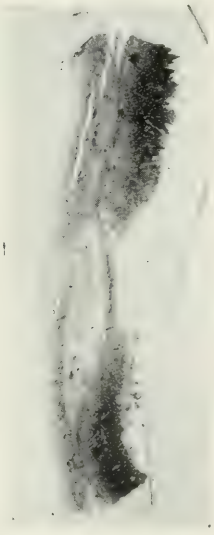


Fig. 15.



(Dr. Y. Miyagawa : An Experimental Study of the Cells of the Mucous Membrane of the Digestive Tract as Toxin or Irriant, etc.)

Fig. 16.

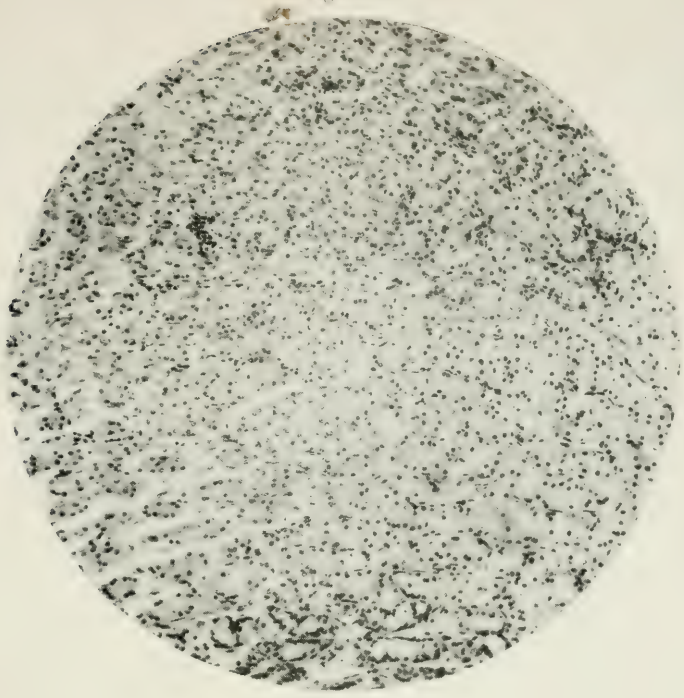
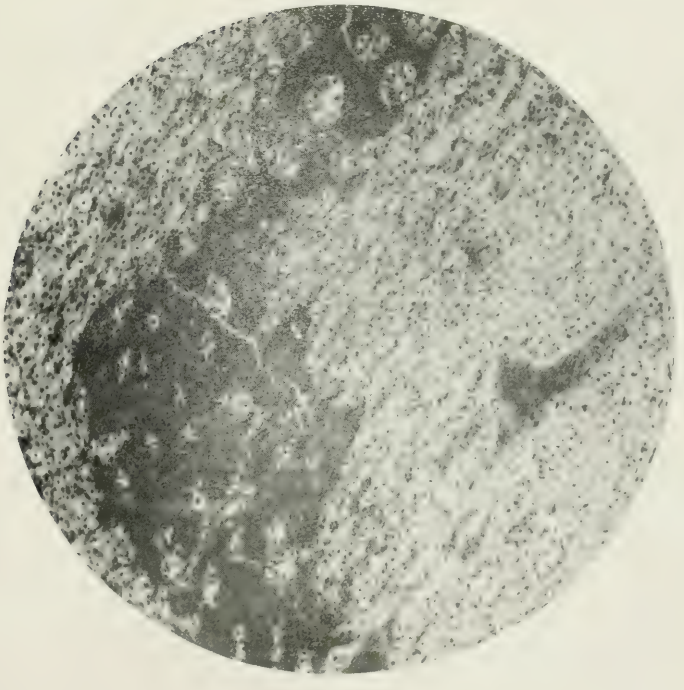


Fig. 17.



(Dr. Y. Miyagawa : An Experimental Study of the Cells of the Mucous Membrane of the Digestive Tract as Toxin or Irriant, etc.)

The Influence of the Mucous Membranous Cells of the Digestive Tract parenterally injected on the Organ or Tissue.

By

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Content.

Chapter I. Introduction.
Chapter II. The purpose and method of experiment, and the observation of general states.
Chapter III. The appearance of blood form.
Chapter IV. The pathologico-anatomical appearance.
Section I. The change of organs due to the injection of the emulsion of homologous gastric mucous membranous cells.
1. Appearance in the case of acute manipulation.
2. Appearance in the case of subacute manipulation.
3. Appearance in the case of chronic manipulation.
Section II. The change in the membranous tissue of the other part that takes place when a part of the mucous membrane of stomach wall is crushed.
Section III. The change of organs due to the injection of the emulsion of heterogeneous gastric mucous membranous cells.
Section IV. The change of organs due to the injection of the emulsion of homologous intestinal mucous membranous cells.
1. Acute change.
2. Chronic change.
Chapter V. Summary and conclusion.

Chapter I.

Introduction.

From an early time, the investigation of metabolism engaged the attention of scholars, and there have been many considerations and observations as to how

the material derived from the outside is assimilated in the living body, But the investigation with regard to what action this assimilated matter will have upon the living body when it is dissimilated and eliminated into the outer world are rather scanty. Much less are the investigations as to how are the cells which are the elements of our body are disposed, or what action they will have upon our body when they are dead. As the matter or fact, the cells of the human body and of other organism are constantly breaking down and being eliminated from the body. Especially, when an organ or tiesue is affected with disease, it can be easily imagined that the breaking down of cells will be unusually large in that part. The components of these dead cells no matter they are of physiological or pathological origin, through the respiratory or excretory organs, are eliminated from the body. But if the components of dead cells are resorbed by the blood vessel and enter the circulation, will they cause no harm or disturbance? Will they have no action upon the organism? This is an important question which has been neglected by old scholars. May parenchymatous living cells are destroyed in an acute pneumonia. But all of the components of these dead cells are not spit out together with sputum. It is a well known fact that a portion of them is resorbed by proteolysis and carried away by the blood circulation. Suppose, on account of various diseases, many of the cells of mucous membrane of digestive tract die at a time. So think that these dead cells will all be digested and fall off from the wall of the digestive tract and be lost in the contents would be looking only one side of the shield. Surely a portion of the components of these dead cells will be in varying quantity resorbed and pass into blood circulation. Whether or not the resorbed components of these cells will be eliminated out without causing no reaction to our body is an important and unsettled question. Recently *Dr. Miyagawa* proposed a hypothesis that, "*if the organ and tissue cells are parenterally injected into an organism, they act upon the homologous cells.*"

He assigned me to investigate what action they will show when the cells of the mucous membrane of the digestive tract are parenterally injected. Under his direction I have made the experimental study of the subject and obtained some results concerning the changes in the organs and tissue of the injected animal. In the study of gastrotoxin, *Dr. Miyagawa* called his attention to the fact that when the cells of mucous membrane of stomach are parenterally injected into the living body, the cells of mucous membrane of stomach will show the change to a certain extent. I have always noticed this fact when I made investigations about gastrotoxin and enterotoxin with *Dr. Miyagawa* and others. Before I will describe my experiments, I shall try to run over the literature concerning this subject and briefly state its resume.

The experimental study concerning the property of the toxin of the cells of organs was started by *Brieger* and *Uhlenhuth*, and later many investigations on various organs and tissues have been made. But most of them were directed to kidney, lungs, liver, testicles and leucocytes. The investigations concerned either

the extract of the cells of above mentioned organs and the neutralization of serum, or cytotoxin, i. e. the serum of the animal immunized with them. Toxicity of cells was discussed on, but there has been no close pathologico-anatomical investigations about the action due to the parenterally injected cells of organs and tissues. In 1912, *Kapsenberg* made a report of very simple experiment concerning the direct action produced by the parenteral injection of kidney and liver cells, without giving detailed account. Recently *Yokomori* and *Okada* of this institute studied about the direct action of lymph-gland and lymph corpuscles. *Kimura* of the same institute about the direct action of kidney and each published the results of very interesting phenomena.

With regard to the direct action of the cells of mucous membrane of the digestive tract, there are only a brief description of *Bolton* and a work of *Miyagawa*. Now dealing with my experiment, the action of the auto-gastrotoxin, as well as the direct action of the gastric mucous membranous cells ought to be considered. I shall, therefore, give the following brief sketch about the histrory of gastrotoxin for the sake of readers.

In 1903, *Théohari* and *Babes* injected into the dog with the goat's immune serum obtained by the subcutaneous injection of the emulsion of the gastric mucous membranous cells of a dog into the goat, and found that the goat serum contained gastrotoxin.

In 1904; *Bolton* produced a powerful cytotoxin, obtained by repeated injections into a rabbit of the gastric mucous membrane of guinea-pig, and found that when such toxic serum is injected into the peritoneal cavity of guinea-pig, necrosis, haemorragic erosion and ulcer were formed in the gastric mucous membrane of the injected guinea-pig. Since then, by his ceaseless efforts many valuable investigations have been published.

But his work was mainly about hetero-gastrotoxin, So far as iso-gastrotoxin is concerned he succeeded in the absorption test, but failed on experiments *in vivo*. Concerning auto-gastrotoxin he hinted at the possibility of the formation of it, but had made no experimental demonstration. In 1920, *Miyagawa* utilizing mass action, by the injection of the serum of rabbit immunized with the gastric cells of a rabbit not into the peritoneal cavity, but under the serous membrane of the stomach wall of the immune or normal rabbit, succeeded in forming a remarkable acute ulcer. In 1922, he and we repeated the same experiments and perfectly demonstrated the formation of iso-gastrotoxin and auto-gastrotoxin and the formation of iso- and auto-enterotoxin. *Miyagawa* and we also made the experimental investigation about specificity of these gastrotoxin.

Thus, the study of gastrotoxin which was started by Theohari and Babes was extended by the hand of Bolton to the fruitful researches in various kinds of heterotoxin and finally brought to the successful experimentation on the formation of iso- and auto-gastrotoxin of *Miyagawa*.

Miyagawa and we who have succeeded in the experiment on the formation

of iso- and auto-gastrotoxin have come fully to think of the possibility of the formation of antigen or auto-gastrotoxin by the cells of gastric mucous membrane which are destroyed by various gastric dieseases in human body and which enter the blood circulation. As above described the experiments thus far almost exclusively related to the study of gastrotoxin. With regard to the research of the changes in the organs caused by the direct action of the components of cells of gastric mucous membrane that are parenterally injected and resorbed into the blood circulation, there is none in the literature on this subject except brief descriptions of *Bolton* and *Miyagawa*. This had induced me to make minutes examination of these changes and to publish the outline of results obtained.

Chapter II.

The purpose and method of experiments and the observation of general states.

The purpose of this experiment is to observe the general states, and to know the pathologico-anatomical change of the animal into whose body the injection of the homogeneous or heterogenous gastric or intestinal mucous membrane is made.

The material of experiment. As the experimental animal, the Japanese rabbit weigning from 2000 to 3000 gram was mainly used. The pregnant or diarrhoetic rabbit, or the rabbit whose organs or general states are not normal was avoided as much as possible.

The material for injection. The gastric or intestinal mucous membrane of rabbit or guinea-pig was used. Of the intestinal membrane the duodenal and the upper jejunal mucous membrane especially were used for the reason of their relatively easy separation or their containing relatively less bacillus. A rabbit or guinea-pig was deeply narcotized with other, and its thoracic and abdominal cavities being opened, the thoracic aorta was ligated and a small perforation was made in its lower part. Into this perforation, a canula through which sterilised salt solution was made to flow, was inserted. Vena cava inferior was out in the thoracic cavity and washed with 2 to 3 litre of salt solution, so that is was made relatively free from blood. Then the stomach and duodemum were taken out and their mucous membrane was washed several times with sterilised salt solution. The mucous membrane was then scratched out and ground up well in a sterilised mortar and adding to it sterilised salt solution or sterilised distilled water, emulsion or autolysate was made. The site of injection. If the autolysate made of 0.5–1.0 gram of the cells of gastric mucous membrane per kilo of the body weight is injected into the blood vessel, the animal may die quite often right after injection. This way of intection is not fitted for our purpose. So I have used mainly intraperitoneal injection.

The quantity of injection. The quantity of injection was various; 0.5 1.0

1.5, sometimes 2.0 gram per kilo of the body weight being used.

The interval and the number of injections. The interval between injections was various; daily, every other day, every third day, once a week being the time of interval. Of the number of injections, the acute manipulation was from one to five times, the chronic manipulation was from seven to twelve times.

The observation of gemeral states. It goes without saying that the extent of disturbance involving the state of whole body varies according to the individuality of the injected rabbit or to the quantity of the injected emulsion of the cells of gastric or intestinal mucous membrane. When one injection of no more than 0.5 gram per kilo is made, the general disturbance of rabbit is relatively slight. Majority of animals were in a state of restlessness after a few seconds oe few minutes from the time of injection, but in a little while, they recovered their liveliness, and showed no changes. When more than 1.0 gram per kilo was used, the rabbit became restless after a few seconds or a few minutes from the time of injection, taking half lying posture, with forelegs stretched forward, and hindlegs layed on the side; the breating was shallow and the heartbeat was quick. After several minutes or several hours, the rabbit somewhat recovered itself from this state but was still impotent and huddled itself in a corner of the cage and had no desire for food and was very slow in movements. But in one or two days it recovered its liveliness. Sometimes the animal showed exsitement following the state of restlesness. In such a case, it is usual that the animal, on the following day, or two days after the state of excitement, returned to the quiet state. Sometimes it happened that the animal suddenly became impotent after the excitment, and was followed by death. When the injection is repeated, the states that has been just described is aggravated and a remarkable decrease of the body weight and final death are usual outcome. The state is not much different in the cases of the injection of the gastric cells of a rabbit and of a guinea-pig. The case of the injection of the intestinal mucous membraneus cells is nearly the same.

Emulsion and autolysate.

Investigators say that autolysate can be more easily absorbed than the cell emulsion and has stronger toxicity. In my experiment the former seemed to be able to cause more serious disturbance than the latter.

The decrease of the body weight and diarrhoea. The decrease of the body weight depends upon the experimental animal, the quantity of the injected material, and the interval and number of injections. When the injection of 0.5 gram per kilo is repeated with 5 to 7 days interval, the decrease of the body weight is not remarkable. Only in a few animals there was an increase of the body weight. When 2.0 gram per kilo was applied at an interval of 1 to 2 days, only one inoculation caused the decrease of 100.0 gram of the body weight after one or two days from the time of injection. In the case of two or three inoculations, the decrease sometimes amounted to 500.0 gram. There is of course more decrease

of the body weight in the animal that suffers from diarrhoea. It is a well known fact that the animal into whose peritoneal cavity the various kinds of organ toxin is injected often suffers from diarrhoea. In my experiment also, especially in the case when the injection was repeated at an interval of 1 to 2 days, this has often been the case. As the most of those that suffered from diarrhoea died, I put them under autopsy no sooner than they are in diarrhoeal state. Immediately before death, convulsion and opistotonus were often noticed.

Chapter III.

The appearance of blood form.

I have examined the change of the blood picture produced by the intraperitoneal injection of the gastric cells of rabbit or guinea-pig.

It is already known that there is a timely change in the total number of leucocytes even in the nomal rabbit, and this physiological change varies according to rabbits. During one or two days previous to the injection, I made several examinations of the blood picture of rabbit every day, and finding that there was not fluctuation, I injected the emulsion into it and then examined the blood from.

The change in the erythrocytes and leucocytes depends upon the individuality of the injected rabbits as well as upon the quantity of the injected gastric cells.

The changes which the heterogeneous and the homogenous gastric cells bring upon the blood picture are not much different.

The erythrocytes. Only one injection of no more than 1.0 gram per kilo may decrease erythrocytes, but in most cases, there was no remarkable change. If the injection of 1.0 gram per kilo is repeatedly made at an interval of one or two days, there is remarkable decrease of erythrocytes and haemoglobin. The state of anisocytosis and polychromasis were more frequentely seen than in physiological states. At the final stage, the appearance of normoblasts was sometimes remarkably noticed.

The change of leucocytes. The injection of 1.0 to 1.5 gram per kilo of gastric cells, no matter heterogeneous or homogeneous the cells may, almost always produced remarkable leucocytosis. The primary leucopenia which occurs after $\frac{1}{2}$ or 1 hour from the time of injection, and which amounts to the decrease by only 1000 or 2000 was not generally remarkable. The temporal relation of the increase of leucocytes was not uniform. But generally it begun at 2 to 4 hours and reached the maximum at 6, 8 and 10 hours and showed 2, 3, 4 times the number of leucocytes before the injection, and this number was maintained from 2 to 4 days. Sometimes even at the 5th day of leucocytosis, the number was still larger than that of preinjection, showing that the leucocytes did not return to normal condition. In 2 out of 13 instances with rabbits, there was no remarkable leucocytosis. In some instances of daily injection of more than 1.0

per 1 kilo the first injection was not much influenced with the second in way of increasing leucocytes. But in other instances, leucocytosis seemed to be augmented with each repetitions of injection. There was no case of leucopenia.

(3) The condition of various types of leucocytes. The most remarkable was the increase of pseudo-eosinophile cells. They increased their relative and absolute mumbers with leucocytosis, and reached their maximum at 6, 8, 10 hours and then their ralative and absolute number gradually decreased, and returned slowly to their normal number from the point of so-called crisis of blood regeneration. The rodlike nuclear pseudo-eosinophile cells may increase even with one injection. But in the case of two or three injections every day, their increase was remarkable after the maximum period of leucocytosis. It was difficult to find out a definite change in the number of mast cells, eosinophile cells and large mono-nuclear cells.

The relative number of lymphocytes decreased with the increase of pseudo-eosinophile cells, although there was no great change in their absolute number. With the decrease of the absolute number of pseudo-eosinophile cells, the lymphocytes gradually increased in their absolute and relative numbers. At 28 to 48 hours from the time of injection, the maximum was reached. At 72 to 96 hours from the maximum point, they usually return to almost normal conditions. Sometimes, they do not return to the normal condition for a long time. With this I shall close my examination of blood form and shall not attempt further description in minute details.

Chapter IV.

The pathological appearance.

Section I.

The change of organs due to the injection of the homologous gastric mucous membrane cells.

1. Appearance in the case of acute manipulation.
2. Appearance in the case of subacute manipulation.
3. Appearance in the case of chronic manipulation. I classified (1) those into which the daily injection, of relatively large quantity of the cell emulsion of the gastric mucous membrane of rabbit was made and which had lasted 1 to 4 days course, under acute manipulation, and (2) those which were injected with the smaller quantity of emulsion at an interval of 1 to 3 days, and which were put under autopsy in 7 to 13 days after 3 to 5 injections under sub-acute manipulation, and (3) those to which 7 to 12 injections of very small quantity of emulsion were made, and which were made to take a chronic course, under chronic manipulation. I shall describe the general condition of these

three cases and state the pathologico-anatomical appearance of each case. The rabbits which were managed with acute manipulation were sure to die or to be on the verge of death after 1 to 3 injections.

In the case of subacute manipulation, the body weight of the injected animal remarkably decreased toward the end, and as the number of injections increased, the manipulation became increasingly difficult. But most of them stood well to 3 to 5 injections. In the case of chronic manipulation, there was some decrease of the body weight after 1 or 2 injections. But later there was no decrease or a slight decrease, sometimes, an increase of the body weight.

(1) Appearance in the case of acute manipulation.

I have had many experimental instance of this case. But I shall describe only following four instances. I made daily intraperitoneal injection of relatively large quantity (1.5 to 2.0 gram per kilo) of the emulsion of gastric cells. In one instance the animal died on the following day after one injection. In two others, one animal died and the other was dying after 2 injections.

In the remaining instance, the animal was dying after 3 injections. In these 4 instances, I noticed that there was a remarkable difference in the pathological changes between those which took very acute course and that which was dying after 3 injections. So I shall make distinction between them and state the pathological changes of each. (a) The rabbit that died on the following day after one injection. I made the intra-peritoneal injection of 2.0 gram per kilo of the emulsion of gastric cells of rabbit into the rabbit. After several hours of restlessness following the injection and the state of excitement that followed, the animal died within 20 hours.

The immediate autopsy after its natural death was neccessary because, if the animals was killed the slight haemorrhage in various organs, especially in the gastric and intestinal mucous membrane or lung may happen and the self digestion of the gastric mucous membrane may accur relatively soon after death.

The gross appearance. Nothing more than a small quantity of free liquid was noticed in the peritoneal cavity. I noticed the solid substance of the injected emulsion attached to the great omentum and the outer wall of the vermiform process and coecum.

There was no suppurative peritonitis. The peritoneum was generally hyperaemic, and the great omentum the and outer wall of intestines, especially the vermiform process and coecum was strongly hyperaemic.

There was a deposit of a small quantity of fibrin in the peritoneal cavity, but no adhesion was seen.

The stomach. The content of stomach was somewhat reduced, and accordingly the size of it was small. A small amount of mucus but no component of blood was seen. There was a slight hyperaemia in the mucous membrane. The hyperaemia was rather remarkable in the fundus part. There was no macroscopically noticeable haemorrhage. The intestines. There was no remarkable

hyeraemia in the mucous membrane of the duodenum and jejunum. There was a strong hyperaemia in the outer wall of the vermiform process and coecum, but none in its mucous membrane. No hyperaemia was seen in the mucous membrane of large intestine and rectum.

The liver. It was reddish brown in color, and its surface was smooth and somewhat soft in consistency. There was a slight hyperaemia.

The kidneys. Its capsule was able to be easily stripped and its surface was smooth. There were hyperaemia, swelling and turbidity in the section, especially in the cortex. Hyperaemia was noticed in the transitional part of the cortex and medulla, but it was slight compared to that of the cortex. There was similar but slighter hyperaemia in the medulla.

The suprarenal gland. Slight hyperaemia in its cortex, but no other remarkable change was noticed. In the heart, lungs testicle and epididymis. There was no remarkable change.

Lymphsystem. There was hypertrophy and swelling of mesenterial lymph-gland. The section showed yellowish gray color. The bone marrow was darkish red and somewhat delicate.

The microscopical appearance.

The stomach. The microscopical small bleedings were found.

The dilated blood vessel filled with erytrocytes were seen in several parts of mucous membrane. As it was put under autopsy immediately after death, no state of self digestion was noticed. There were mucus and cell detritus on the surface of mucous membrane.

The change of gland cells was very remarkable.

(a) The gastric fundus gland. The most remarkable change in the gastric fundus gland is that of parietal cells. The change of principal cells is more slight. Physiologically, the pareital cell is polygonal or roundish, and its protoplasm has a relatively large nucleus, in the centre of which is a round nucleolus. The nucleus is stained remarkably well with anilin dye. Generally, there is very often vacuolar formation in the parietal cells when the digestive process in the stomach is going on vigorously. For there is always more secretion of gastric juice than could be eliminated by intercellular secretory capillary tubuli. In the present instance, the aragement of parietal cells was very irregular, and some of them were so located as if they were pushed out into the cavity of gland and some were located far apart on the outer side. Their shapes were multiform ; being round, long, or oblong. Of the protoplasm, some had nearly normal distribution of granules, but many others had very irregular distribution of them.

The size of granules was various. There was a vacuolar formation in a high degree in the protoplasm. Besides, plasmolysis or plasmoptysis were seen in many parts. The state of staning was various.

Some of them stained very deeply, and that which shows scarlet color by haemotoxylin-eosin staining is especially remarkable.

The nucleus of that which has such protoplasm was usually pycnotic. The protplasm of some cells decreased their staining capacity.

The change of nucleus was very remarkable. Most of them were no more round shaped as in the normal state; the shapes being zig-zag, spindle, line, sometimes pyramidal, rhombus, or angular. They stained very deeply and it was very difficult to see the inner structure.

Side by side with those nucleus which showed pycnosis, there were some which were in the state of karyolysis and karyorhexis. Some have lost nucleus entirely, and became structureless. The principal cells. The structure of principal cells is plainer than the parietal cells. They are cubical or columular in shape and have globular nucleus in their protoplasm. At the time of digestion, they become like pyloric gland cells dark in color on account of vigorous secretion. The nucleus is eccentric located near the base in the rest time, but moves to centre at the time of digestion.

The ganules of principal cells are usually small and slender, and dark colored near the base, bat become courser, larger, and plainer as they approach the cavity of the gland. In this instance, the granules were uniformly atrophic and darkish, or remarkably plain showing the vacuolar formation. The change in the nucleus of cells was manifold as in the case of parietal cells. But the number of degenerated cells is not so large. Those which showed pycnosis had various shapes and it was difficult to distinguish their structures. There were disappearance and detachment of principal cells. A relatively slight change was noticed in the superficial cells of mucous membrane. Specially remarkable to the decrease of staining capacity of protoplasm. In the interstitial tissue between glands no hypertrophy and the infiltration of round cells were noticed. I could see, however, dilated capillaries filled with blood corpuscles, and small bleedings in several parts and some extravasation of blood corpuscles out of the capillary vessel.

There was no changes in the submucous muscular layer and the muscular coat except a slight hyperaemia. By fat staining we could find minute granules filling the parietal cells of the gland cells.

But they are also found in ordinary physiological state and besides, the size of granules in present instance was relatively uniform.

(b) The case of the animal to which the daily injection of relatively large amount of emulsion was made, and which was found dying on the 4th day after 3 injections.

I injected every day 1.5 gram per kilo of the emulsion of homogeneous gastric mucous membrane into the rabbit and found that the animal was on the verge of death on the 4th day after 3 injections. In 3 days the rabbit showed the decrease of about 500 gram of the body weight. As soon as it died on the 4th day, I put it under autopsy.

The macroscopical appearance.

There was a small quantity of free liquid in the peritoneal cavity. There

was a general hyperaemia in the peritoneum.

A slight adhesion in the outer walls of intestines was seen, but the detachment was very easy.

The stomach. Its content was greatly reduced and the stomach was not more than half the size of the normal one. There was a large quantity of mucus and some blood intermingled with the contents, showing darkish color. The mucous membrane was somewhat hyperaemic.

There were macroscopical haemorrhages or the haemorrhagic erosions here and there. The bleeding spots were in the gastric fundus, gastric body and isthmus. The haemorrhagic spots which were pretty numerous were the size of pin-head; some were larger and some smaller than that. The intestines. In the duodenal and jejunal mucous membrane there was a slight hyperaemia but no remarkable catarrh. There was no marked macroscopical change on the surface of the mucous membrane of the ileum, coecum, large intestine and rectum.

The liver. The liver was rather anaemic and yellowish in color. The surface was smooth. The section was also poor of blood and yellowish. The kidneys. They were also anaemic and the cut surface was yellowish. This change is most remarkable in the cortex, and apparently reminds us of fatty infiltration. In the suprarenal body, there was hypertrophy of the cortex, slight hyperaemia, and swelling and turbidity of the medulla. There was no marked change in lungs. There was sweeling and hypertrophy of mesenterial lymph-gland, and its section was yellowish gray.

The bone marrow was darkish red, and delicately gelatinous. There was little hyeraemia and slight hypertrophy in the spleen.

The mieroscopical appearance.

The stomach. In this instance, the change of the gastric mucous membrane was greater compared to that of the former instance caused by one injection. Hyperaemia too was stronger; and there were haemorrhagic erosions in many parts. Besides, there were erosions without bleeding. In some places, of glands, I could not recongnize the gland cells on account of the uniform necrosis of them in that part. Sometimes the hyaline embolus was found in the capillaries. The cause of such necrosis would naturally be thought to be the initial haemorrhage followed by the death of the epitherial cells. But the haemorrhagic erosions, which were seen in the present instance in which there was no remarkable hyperaemia and there was necrosis of the gland cells in many places which have no relation to the bleeding parts, should rather be considered to be the case in which necrosis primarily occured in a part of the mucous membrane of stomach which receiving an action of the digestive fluid, brought forth the haemorrhage. More so because there was present also non-haemorrhagic erosion.

Again we must not overlook the embolus of small blood vessels as a cause of such necrosis. I have made a close examination of the relatively remarkable of the necrotized parts. There was almost no change in the muscular layer of

mucous membrane. In the glandular bottom or its adjacent part, the gland cell had relatively normal structure. But as it approached to the mucous membranous surface, the change became very great, and it was difficult to recognized the structure of the gland. The distinction between the principal and parietal cells was not clear. In the part near the mucous membranous surface, there was an accumulation of nucleal detritus, and on its side, a lump of degenerated proteids. The large necrotic patch showed a shape of triangle whose apex is in the deeper layer.

The small necrotic patch had nearly all cells of gland detached off, the cavity of gland blocked, only base membrane as what remained of the gland. In the parts other than necrotic patch, the degenerative process both of the principal and parietal cells is very striking, and the degenerations of protoplasm such as vacuolar formation, plasmolysis, plasmoptysis, and pycnosis, karyolysis, and karyorhexis of the nucleus of cells were seen here and there. The staining capacity of nucleus abnormally diminished. Sometimes the degenerated protoplasm which has entirely lost the nucleal substance and was surrounded by the cell membrane was often noticed. In the part above the neck of gland, nearly all the cells showed such degenerative changes. There was also the degenerative process of various extent in the epithelial cells of mucous membrane.

The surface of mucous membrane was covered with a large quantity of mucus and had much cell detritus on it. In the interstitial tissue between glands, severe hyperaemia and haemorrhage were seen in several parts, but there were almost no hyperaemia and haemorrhage in other parts. No infiltration of round cells was noticed. In that part of the interstitial tissue between glands where severe necrosis took place, there appeared young fibroblasts. In other parts, there was no hypertrophy of the interstitial tissue between glands.

The intestines. There was a slight hyperaemia, but no haemorrhagic erosion. The change of goblet cells is interesting. The granular arrangement of their protoplasm was very irregular and some had very large vacuol. The change of nucleus is very marked, taking various forms such as angular, spindle, and sometimes with entire disappearance of it. There was a noticeable decrease of large granules of Panet's cells.

The microscopic appearance of other organs in (a) and (b) cases are described together.

The kidney. In the case in which the injection of a large dose of the emulsion of the gastric cells was made and the animal was dying in a day or two, there was marked changes in the kidneys. The main changes were hyperaemia and haemorrhage. They were most remarkable in the cortex and the transitional part of the cortex and the medulla. But they were observed also in the medullar sbustance. The hyperaemia was specially remarkable in the glomerulus and its surroundings. In the animal which was dying on the fourth day after three injections and which was put under autopsy on the same day, the hyperaemia of the kidney was very slight.

The glomerulus abounded with erythrocytes, and a little bleeding was noticed in it. Erythrocytes were sometimes found between the epithelial layers of Bowman's Capsules. The hyperaemia and haemorrhage of the interstitial connective tissue were remarkable both in the cortex and the medulla. There were extravasation of blood corpuscles in the convoluted tubules, which accumulating in a place formed erythrocyte casts. The change in the epithelial cells of the uriniferous tubules was relatively slight, but in the glomerulus there were sometimes a vacuolar formation and turbidity of the endothelial cells, and pycnosis of the nucleus. In the convoluted tubules there were more or less turbidity and sweling, but very little vacuolar formation and detachment of epiterial cells. The change in the intercalary portion was very slight. There were similar changes in the Henle's loop and the collecting tubules, namely turbidity and swelling, vacuolar formation in the epithelial cells.

There were a few caryorhexis and caryolysis of nucleus. In the cavity of tubules, something like erythrocyte casts, hyaline casts and granular casts were observed, but none of epithelial cellular cast. Some detached epithelial cells were seen in the cavity of tubules of various parts, but their number is very small. There was seen no such necrotic patch in a certain part ss was seen in the case of the injection of kidney cells emulsion. But in the case of daily injection of a large quantity of the emulsion of gastric cells in which the animal was on the verge of death on the fourth day after three injections, the change was somewhat different from what has been just stated above. The most remarkable differences were firstly the degree of hyperaemia and haemorrhage, and secondly, the degree of the degenerative process in the epithelial cells of uriniferous tubules. In the present instance, the hyperaemia and haemorrhage were slighter but the degenerative change of parenchymatous cells was much greater. In this case, there were more erythrocytes in the glomerulus than in former case. Also there were many lymphocytes and other round cells. The epithelial cells showed some degenerative changes. The degenerative process in the Bowman's Capsule was sometimes noticed. The change in the convoluted tubules was especially remarkable, and its turbidity and swelling, the irregular arrangement of granules, plasmolysis and plasmoptysis were all greater.

Rarely pycnosis and caryorhexis of nucleus were noticed. Closely examined, the disappearance of nucleus, falling off of epithelial cells into the cavity of tubules were observed. But no necrosis of the parenchymatous cells of kidneys seen in the case of the injection of kidney emulsion was seen. The straight tubules, and Henl's loop showed turbidity and swelling and plasmolysis in their epithelial cells, and sometimes pycnosis and caryorhexis of nucleus.

The detachment of epithelial cells, epithelial casts and nuleal casts were sometimes found. But these changes were much slighter compared to those which were caused by the injection of kidney cells. So far as lipoid staining was concerned, in the formar case in which the animal died on the second day, in

spite of very slight degenerative change in the epithelial cells, there was fairly strong fatty infiltration. This was specially marked in the ascending limb of Henle's loop and its basal part was often found to be filled with fat globules. Small granules were found also in the descending limb of Henle's loop. In the convoluted tubules there were infiltration of varying degree in different parts. In the extreme case, fatty droplets of different sizes were seen crowding in the basal part. In the slighter case, only a few minute granules were noticed here and there. There were relatively few fatty infiltration in the intercalary portion. In the glomerulus, the marked fatty globules were noticed. This was specially the case in the convoluted tubules. Also the fatty globules were seen in the endothelial cells of blood vessel and the interstitial tissue. In the casse in which the animal was dying on the fourth day, there were seen the fatty granules of various sizes in the epithelial cells of the convoluted tubules, especially in its basal part. The fatty globules were found also in the cavity of convoluted tubules. There was an interesting contrast between those which had relatively large amount of fatty substance and those which did not have. In the Henle's loop, the fatty content was larger than in the normal rabbit, but it was less compared to that in the convoluted tubules generally. There was almost no fat in the glomerulus. The liver. In the animal into which a large dose of gastric cells was injected and which was dying on the following day, the change of the gastric mucous membrane was more remarkable compared to the change of intestines and kidneys as stated before. On autopsy which was mede on the following day or the next to the following day, the main change was found to be the hyperaemia in the liver, and the parenchymatous change to be relative slight. There were fairly strong hyperaemia and the dilatation of the capillary blood vessels and the central vein. But there was no bleeding. There was a remarkable hyperaemia in the blood vessel of liver lobules, specially in the portal system. The vacuolar formation of the parenchymatous cells of liver were found here and there. But this kind of change was very slight compared to the change which was seen in the animal which was put under autopsy on the fourth day. Closely examined, various degenerative changes as pycnosis and caryorhexis of nucleus, extreme diminution of pigments, and faint staining of relatively large nucleus in which vacuoles were contained, were seen. But all these changes were slight when stained with sudan III. There were seen smal fatty globules in the liver cells. They were more remarkable near the central vein.

A few fatty granules were seen in the connective tissue of Glisson's capsule and the endothelial cells of bileduct. The most remarkable are the endothelial cells of blood vessel and Kupfer's cells which were almost filled with fatty granules. This was specially the case in the central vein and its adjacent parts. But in the case of "K", the degenerative process of the liver cells was remarkable, and hyperaemia and haemorrhage were not so remarkable. The most striking change in the liver is the formation of vacuoles of various sizes in the liver cells.

Sometimes the whole of the liver cells were occupied by them, and the degenerated nuclei of liver cells looked to be pressed to one side. There were some vacuoles also in the liver cells. The appearance of such vacuoles was relatively remarkable in the peripherial zone of liver lobules. The change of nucleus was relatively small. Besides pycnosis, caryolysis, and caryorhexis which were often seen, there were extreme diminution of the chromatin of nucleus.

In the protoplasm, a remarkable change in the line of plasmolysis or vacuolar formation, sometimes bare remnant of protoplasm from which the nucleus had entirely disappeared was observed. The appearances resembled the degenerative processes of liver seen in the case of the parenteral injection of the liver emulsion. But the local necrosis of the liver parenchyma noticed in the latter case was not recognized in the present case. By staining with Sudan III, it was found that the lypoid granules of various sizes were present in abundance especially in the peripherial zone of the liver lobule, and those which were found near the central part were rather of minute sizes and not so numerous, and scattered amoung them, Kupfer's cells having large granules were often seem. From this appearance, the distribution of fat would look like that of fatty infiltration.

But the cells whose nuclei underwent a remarkable change suggested the fatty degeneration. Hence it would be reasonable to conclude that the appearance was due partly to the fatty filtration and partly to the fatty degeneration of the parenchymatous cells. There was almost no infiltration of round cells in the interlobular tissue, neither a hypertrophy of the connective tissue.

The lungs. In the animal which died on the following day as the result of the injection of a large dose, there were seen besides hyperaemia in lungs, small bleedings here and there. Microscopically some blood corpuscles were found in the pulmonary vesicle. But there was no other marked change. In the animal which took three or four days course with daily injections, there were though small in extent atelectasis here and there. In such an affected part, there would appear a large amount of cells in the alveolar walls, and the alveoli may be blocked. Of the cells seen in such aveolar walls, the most numerous are the round cells, large mononuclear cells, lymphocytes, and mast cells. Not a small number of pseudo-eosinophile cells were also observed. Iu such a part, the leucocytes and erythrocytes, besides large mono-nuclear cells, were often noticed in the pulmonary visicles.

In other words, the change in the lungs compared to the change in lungs of the animal into which the parenteral injection of the emulsion of lung is made is very slight.

The heart. In the animal which died on the following day on account of a injection of a large dose slight turbidity in the muscle cells and the engorgement of blood vessels were seen. By fat staining, we found in varying quantities, in different parts, the presence of fatty granules of various sizes all over the organ. Fatty granules were found in the endothelial cells. In the animal which was

put under autopsy after 3 to 4 injections, there was a slight hyperaemia besides turbidity and swelling of muscle cells.

The suprarenal body. In both cases of one injection of a large dose and of the autopsy after 2 to 3 injections, there was slight hyperaemia in the cortex. The change of parenchymatous cells was marked in the reticular layer and the glomerular layer. In the one and same gland, there were side by side with almost normal cells the cells which had the nucleus showing pycnosis and which were stained deeply with eosin. The fatty content was relatively small in those cells which showed such degenerative processes. Some hyperaemia, turbidity and swelling in the medulla were seen. But the change of this sort was slighter compared to that in the cortex. The spleen. In the animal which died on the following day after a large dosed injection, there was no remarkable change besides a hyperaemia in spleen sinus. But in the animal which was put under autopsy after 3 successive injections, there were seen marked erythrophagy here and there besides a slight hyperaemia. Accordingly a large amount of haemosiderin were observed there. There were many histiocytes which were filled with yellow pigments.

The bone marrow. In the case of the animal which was put under autopsy after the injection of a large dose, there was alight hyperaemia in the bone marrow. But there was no leucocytosis. In the case of autopsy after two or three injections, there was a marked myelosis in it. Specially remarkable and numerous are the pseudoeosinophile cells. There was an increase also of myelocyte and myeloblast. There was an evident appearance of myelopoesis. The increase of the giant-cells of the marrow was also remarkable.

Lymphgland. In both cases of one large dosed injection and of two or three injections, there was very little change. Only change noticed was a hyperaemia in lymphsinus.

(2) The appearance in the case of subacute manipulation. The emulsion of the gastric cells of a normal rabbit was injected into a rabbit according to the method above described. In first instance, 4 injections were made every other day and the animal was killed on the 8th day. The quantity of emulsion used was first 0.5 gram and it was gradually increased to 1.0 2.0 and 3.0 gram.

In the second instance, four injections were similarly made.

The quantities used were 0.5 1.0 2.0 and 3.0 gram. The animal was killed on the 11th day. In the third instance, the similar four injections were made, and the doses were 1.0, 1.5, 2.0 and 3.0 gram. The animal was killed on the 7th day. In the last and fourth instance, using 2.0 gram each time, and with an interval of two days, the injection was made, and the animal was put under autopsy on the 8th day. The naked eye and histological appearances of these four instances almost agreed with those of the animals which were put under autopsy in two or three days after one or two injections.

But closely examined, some differences were found. There were some

points of agreement between the change in these instances and that which was produced by the chronic manipulation, which will be discussed later. I shall now take up the appearances of these animale in a bundle and discribe them as the intermediate form. In these experiments, the experimental animals all showed the decrease of thd body weight by 200 to 400 gram.

The macroscopical appearance.

There was a general hyperaemia in the peritoneum. Solid substances of injected emulsion were seen in various parts of the peritoneal cavity. A remarkable hyperaemia was seen in the great omentum.

Much deposit of fibrin, and the slight adhesion in the intestinal walls were noticed.

The stomach.

Generally there was a decrease of the stomach contents, and among the contents there were a little quantity of liquids. The content were abundant in mucus and intermingled with blood components. There was strong hyperaemia on the surface of mucus memberane, especially in the fundus part. In the two instances out of the four, the haemorrhagic erosion or small ulcer was found. In one instance, they were found along the greater curvature in the gastric fundus and the gastric body. They were of the size of pinhead or smaller and the number was more than 10. In another instance, they were located in the gastric body and the gastric fundus. Their size was larger and their number was relatively small. There were always catarrh and generally slight hyperaemia in the intestines, duodenum and jejunum. In the 3rd instance there was strong hyperaemia and many small bleeding spots were seen in duodenum. There was little hyperaemia in ileum.

Hyperaemia was seen in the serous membrane of coecum and large intestines. But on the surface of the mucous membrane there was no macroscopical changes. The liver. Its surface was smooth and there were hyperaemia and swelling. The section was darkish red and rich in blood. There was no hypertrophy of the interstitial tissue of lobules. The kidneys. No change on the capsule. There were hyperaemia, turbidity and swelling in the cortex. A similar but generally slighter change was seen in the medulla. It was usual that the cortex of suprarenal body was generally slightly congested. But in one instance, the volume of the suprarenal body was very large and both the cortex and medulla were darkish red. The spleen. There was no remarkable change except slight hyperaemia. The lymph gland. The mesenterial lymph gland was always hypertrophic and darkish gray in color. The bone marrow was darkish red and sometimes very soft. The lungs and heart. There was no marked change besides a slight hyperaemia. Sometimes small bleedings were seen in the lungs.

The microscopic appearance.

The stomach. (1) The gastric fundus gland area.

There was a hyperaemia of fairly high grade. The haemorrhagic spots of

the size of pin-head were seen in various parts. They were not yet digested and did not form erosion. Histologically, there were two kinds of tissues which were necrosed and formed erosion. The one was the typical haemorrhagic erosion, and the other attended by no bleeding, and sometimes by no hyperaemia. This necrotic patch, as stated before, showed the form of equilateral triangle whose apex was in the deeper layer of mucous membrane. But in some, the relation was not clear, or there subsisted an oppoite relation. In such non-haemorrhagic erosion, the hyaline embolus were seen in the capillary blood vessel of mucous membrane. We might suppose as a cause of the formation of haemorrhagic erosions that the injected cell components of the gastric mucous membrane through blood circulation may have direct action upon the cells of gastric mucous membrane and produce their necrosis, and the digestive liquid acting upon the necrosed tissue may produce erosion and the secondary haemorrhage. There was something in the appearance of present instances which confirmed this idea. The formation of such necrotic patch and erosion was seen also in the pyloric gland area. There was more or less infiltration of round cells in the interstitial connective tissue between glands and the proliferation of the interglandular tissue. This appearance was much different from that of the instances in an acute manipulation and closely resembled the chronic change what will be discussed later. The infiltration of round cells was specially remarkable in some part of the interstitial connective tissue of glands. In some parts very little and in other parts but no infiltration was noticed. In the parts where the infiltration was striking, there were many fibroblasts, some of which already became spindle shaped, and the others became real connective tissue. Perhaps there is no need of detailed explanation of the orgin of the hypertrophy of connective tissue. Clearly it is the consequence of the atrophy and necrosis of the cells of gland. There were various degenerative changes in the epithelial cells. The most common changes were the diminution of the staining capacity. In some part, the epithelium almost entirely fell off. In other parts, the epithelial cells were relatively normal. There was a large quantity of mucus on various parts of the mucous membranous surface. The surface of the mucous membrane was covered with detritus of gland and epithelial cells. Often a few blood corpuscles and pseudo-eosinophile cells were found in it.

The change of gland cells was essentially the same as that described in Section I. The most remarkable is the change in the parietal cells. What I want to decribe is not such changes as seen in the necrotic patch but the change in the gland cells in the part where no haemorrhage was observed, and where only hyperaemia was barely noticed. Specially remarkable appearance in the parietal cells is the change in their staining capacity.

Their staining with aniline, especially with eosine was various and they which stained very deeply usually contained pycnotic nuclei. Sech cells were found scattered here and there, or in groups. Closely examined, the protoplasm of

parietal cells showed plasmolysis, plasmoptysis and vacuolar formation. Generally they which stained faintly contained the large nucleus losing its chromatin. Sometimes, the nucleus entirely disappeared and protoplasm only remained there structureless. Many of them lost their original shape and became rectangular, zig-zag, ragged, irregularly curved, or gourd shaped.

These nuclei were in pycnosis, stained deeply and were structureless. There were nuclei which unlike those that have been mentioned, became enlarged and lost the chromatin and still remained in the degenerated protoplasm. Not a few of them had vacuoles. The position of such degenerated nuclei in the cells were very indefinite ; being on one end, or one side, or coming out of the protoplasm. The location of the degenerated parietal cells in the gland did not always take one sided position as seen in ordinary, physiological condition. Sometimes they were seen so far apart that one would suspect the loss of connection in the gland, or entering the file of principal cells, or still deeper approaching the inner cavity, or finally falling in the cavity of gland. By staining granules according to Altmanschridde's method, the arrangement and size of granules were found to be remarkably different from those in the physiological condition. But as the matter of fact, there were not a few cells which did not show any such changes. The change in the principal cells was similar to that of the parietal cells. In the protoplasm there were many cells which showed vacuolar formation, plasmolysis and plasmoptysis. Pycnosis, caryorhexis, and caryolysis were seen in the nucleal substance. The nucleus of cell which was pycnotic showed also various forms which were seen in that of parietal cells.

Examined by granular staining, fairly large number of cells which have lost their physiological structure was found. The gland in which seriously degenerated cells were crowded was remarkably atrophic and accelerated the hypertrophy of the interstitial tissue between glands. In this way, the tissue of the gastric mucous membrane was generally thin stiff and atrophic.

The phloric gland area.

The change of the epithelial cells and the mucous membranous surface was the same as the changes of those in the fundus gland area. I was able to find a slight hypertrophy of the interstitial connective tissue between glands in this part. The change in the gland cells was degenerative process. In some it was strong, in some slight, and in some there was no change at all. In the degenerated cells, pycnosis of nucleus was specially remarkable. Of the nuclei, there were some which contained vacuoles, and others which enlarged and whose staining capacity greatly diminished. In the protoplasmic substance, there were plasmolysis plasmoptysis and vacuolar formation. By granular staining I was able to find the irregular arrangement and other abnormalities of the granules in the cells of pyloric gland.

By fat and lipoid stainings I found that some of the cells in the fundus and pyloric glands, contained fairly large number of minute granules. Glycogen staining

would show that some what large quantity of it was contained in the cells. But in many of them, no granules were found. This must have been due to the fact that the animal lost the desire for food and was emaciated accordingly. From those appearances we have to conclude that these are the degenerative process and necrosis of the cells of gastric mucous membrane in the fundus and the pyloric glands, and the similar change in their epithelial cells. Of these changes in the gland cells, the most remarkable is that of the parietal cells. This may be conceived to have originated the change of the components of gastric jujce, especially of the content of hydrochloric acid in the animal. Somewhat strong hyperaemia was seen in the blood vessels of the interstitial connective tissue between muscles and the lower layer of mucous membrane. But there wan no haemorrhage at all. Only in one instance there was some variation in staining of muscular fibres. By fat staining, I was not able to recognize fat globules. Such an appearance is what we often see in normal animals.

The change in other organs was nearly alike to that described in the first chapter and will be described here briefly,

The kidneys.

Hyperaemia and small haemorrhage were noticed in the cortex and medulla. The change was greater in the cortex. There was a degenerative change in the epithelial cells of the straight and convoluted tubules. The grade of the change was higher than that in (a) of the Section I, but slighter than that of the experiment (b). The noticeable point in this case was that there was a large number of pseudo-eosinophile cells in the glomerulus. In one instance, I noticed very interesting degenerative change of intercalary portion. In this instance, the epithelial cells of intercalary portion stained deeply in the eosine, and the nucleus showed pycnosis. There was no deposit of lipochrom. There was a slight hypertrophy of the interstitial tissue. By fat staining, I found somewhat large quantity of the fatty infiltration in the cortex and medulla. The fatty infiltration was seen in the ascending limb of Henle's loop in a larger quantity than in physiological condition.

The liver. The change of liver was about the same as that which was described in Section I. Generally there was a slight hyperaemia. The cells of liver showed changes varying from a serious to a relatively normal states. The vacuolar formation in protoplasm was not so marked but the irregular distribution of the granules plasmolysis and plasmoptysis were also observed in degenerated cells. In this instance, there was a great variation in the sizes of nuclei. Of the larger ones, much nuclear chromatin was found in peripheral part, and the central part was transparent, in which was seen a remarkably enlarged nucleolus. Such large nucleated cells were mostly seen in the peripheral part of liver lobules. There were a crowd of liver cells which were deeply stained and atrophic, containing pycnotic nuclei. Of the smaller nuclei they were not stained well, showing barely their out lines. Caryorhexis and caryolysis of various degrees were noticed. The change of liver in other three instances was relatively slight

and nothing except hyperaemia and a slight change of liver cells was seen. In one instance a slight hypertrophy of the interlobular connective tissue was suggested. By fat staining minute granules were seen more or less throughout liver cells. The suprarenal boby. The change is about the same as that in the acute case. Generally slight hyperaemia in the cortex was seen. Some of the cells of gland, though not so many, stained deeply and had pycnotie nuclei. The lungs. As in the acute case, hpyeraemia and small haemorrhagic spots were found. Atelectasis appeared in many places. In that part, often round cells, leucocytes, and erythrocytes were seen in the wall of pulmonary vesicles. These cells were often found in the alveoli. The heart. Besides a slight hyperaemia, minute fat granules were observed in several parts. The spleen. There were a strong hyperaemia and the dilatation of spleen sinus. Erythrophagy was often seen. Haemosiderin deposit was seen more than in normal state. In one instance, a remarkable leucolysis was recognized. In this instance relatively large number of pseudo-easinophile cells was found in the follicle. The most of their nuclei showed caryorhexis. The size of the granules of pseudo-eosinophile cells was indefinite and they were seen scattered outside of the membrane of cells. The lymphgland. There were slight hyperaemia and haemosiderin deposit in the mesenterial lymph gland. Many pesoudo-eosinophile cells were seen in the lymph-sinus. Erythrophagy was sometimes seen. The bone marrow. There was slighty hyperaemia and haemosiderin deposit was seen here and there. In all instances myelosis was observed. There was a special increase of pseudoeosinophile cells. There was also an increase of myelocyte and myeloblast.

A remarkable increase of megacaryocytes was also proved. The phenomenon of myelosis was higher in grade compared to that of the animal which died on the following day after the injection of a large dose, and was slighter compared to that of the animal into which the successive injections of a large dose for several days were made.

(3) Appearance in the case of cronic manipulation.

I shall now describe the change in the cells of the gastric and intestinal mucous membrane and in other organs produced by the intraperitoneal injections of the gastric cells during a period of long time. This kind of experiment is usually made in the experiments on the formation of gastrotoxin. The experimental animals accordingly were very numerous, but I shall describe here the instances of a few animals. The method of experiment was as follows. The gastric cells of the rabbit was ground up in a mortar, and an emulsion in salt solution or autolysate in distilled water has been made out of it. Into the peritoneal cavity of a rabbit, from 7 to 12 weekly injections of this solution were made each consisted of about 0.5 gram of cells per kilo. In a week from the time of last injection the animal was killed and put under autopsy. The naked eye and histological examinations were made.

The macroscopic appearance.

Usually the animal which passed a week after the last injection showed slight hyperaemia in the peritoneum. Injected emulsion substance which was wrapped in the membranous substance was seen attached in several parts of the peritoneal cavity. The contents of stomach were somewhat reduced. Much mucus also was recognized.

Often several small ulcers were seen especially in the pyloric gland area. There were not many haemorrhagic erosions in the fundus and other parts. Slight hyperaemia was seen in the intestines, kidneys, liver and other organs. But no other remarkable change was noticed.

The microscopic appearance.

Microscopically, there was a remarkable degenerative processes in the cells of mucous membrane of the stomach.

The interstitial tissue between glands was hypertrophic in all instances. In a more violent case, a remarkable atrophy of the mucous membrane was recognized. But in some animals, there was no strong hypertrophy of interstitial substance, only change being the presence of a small number of round cells in the increased connective tissue. The specially remarkable was the change of parietal cells. The staining of protoplasm was very uneven. Some cells stained very deeply, the other ones stained but faintly. Besides, vacuolar formation, diminution of staining capacity, irregular arrangement of granules appeared in various forms. The fatty content was more than physiological in some cases. The forms of degenerated cells were also manifold. The arrangement in the gland was irregular; some located very near to the cavity, some pushed out to the external side, some placed side by side with the principal cells, some fallen in the cavity of the gland. Physiologically, the parietal cells are most found in the neck of gland. But in this instance they seemed to be located in the duct rather than the neck, and many of them appeared to have been pushed out to the external side. Generally, the number of parietal cells seemed to have decreased. Evidently the change of these parietal cells has some relation to the constituents of gastric juice.

The pathological change in the nucleus also was marked. Pycnosis, vacuolar formation, diminution of pigments were the most common change. Especially of the cells whose nuclei were pycnotic and showed abnormal forms, the protoplasm always stained deeply with eosine staining and was clearly distinguished from that which stained faintly. The positions of the nuclei of the degenerated cells in protoplasm were various too. The manifold degenerated nuclei were present side by side with the apparent physiological ones, displaying a variegated appearance under microscope.

A similar change in the nuclear substance and protoplasm of principal cells was observed. There was detachment of gland cells.

When we apply granular staining method of Altmanschridde to our examination, the changes of both the principal and parietal cells became all more

remarkable. There was generally more fatty granular content than in physiological condition. As stated before, many detrital cells were seen intermingled with mucus in the surface of mucous membrane. Among them, there were parietal cells, principal cells and sometimes a large number of leucocytes.

There were various kinds of degenerative processes in the epithelial cells of mucous membrane. In a word, the change in this experimental instance was very slight compared to the acute change produced by the parenteral injection of the emulsion of the gastric cells. (Ref. 1.2.) In the blood vessels of the mucous and submucous membrane, there was often slight hyperaemia, but no haemorrhage.

The pyloric gland area.

There was no hyperaemia in this part. The mucous membrane was thick and stiff. Physiologically, the mucous membrane of this part is more stiff compared to that of the fundus and the gastric body, and adheres firmly with the lower layer. But in the animal which has received the manipulation, this was specially the case. To speak of the microscopic change of the mucous membrane of this part, the necrosed and detrital cells on the surface of the mucous membrane was less than in the fundus gland part. Neither was there much mucus. But there was relatively large number of parenchymatous cells which have undergone various kinds of the degenerative changes and which had remarkably large fatty content. The change seen in nucleus and protoplasm nearly agreeds to that of the principal cells of the fundus gland.

The cells which were subject to these degenerative changes were not very many and present side by side with the perfectly normal cells. The hypertrophy of the interstitial tissue between glands specially attracted my attention. It was found in any place in two gastric glands area. But it was most remarkble in the pyloric gland area. Generally where the change of gland tissue was great, there the hypertrophy of interstitial substance was marked. Among the hypertrophic connective tissue, there were the young fibroblasts. There was often a little infiltration of the round cells in the interglandular tissues. It seems that such necrosis of gland cells in this case as would appear as the result of accute irritations succesively appeared rendering the perfect regeneration impossible, and accelerating the hypertrophy of interstitial substance, and atrophy of the mucous membranous surface. Contrasting this appearance with the acute change, the following points of difference were discerned. (1) In the animal which received the chronic manipulation, there was no such remarkable hyperaemia or haemorrhage as seen in the acute change. (2) There were fewer number of the haemorrhagic or non-haemorrhagic erosions or necrotic patches of gland cells. But there was the formation of ulcer. (3) The last mentioned change was found also in the subacute manipulation and therefore may not be regarded as a point of remarkable difference. But no matter whether the case may be acute or chronic, there were various kinds of degenerative processes in the parenchymatous cells of glands. These changes which would ultimately lead each cell into necrosis were common to both.

In other words, both were essentially the same in genesis of changes. But in the acute case there was no hypertrophy of interstitial substance. Only in that which was described as subacute type there was seen a slight change (Ref. (2)). It was therefore natural that the atrophy of the tissue of mucous membrane occurred in connection with the change in chronic manipulation. So sum up, there is a remarkable change in the tissue of gastric mucous membrance under the above described manipulation, no matter acute or chronic the manipulation may be. This change will bring the degeneration and necrosis in a large or small scale, or in some individual cells. But there is almost no noticeable change in the submucous layer and the muscular layer and others.

Appearance of other organs.

The intestines. Except haemorrhage and hyperaemia, we might say there was almost no change histologically. But close examination showed that there was somewhat strong degenerative process in gland cells, especially in the goblet cells, and often a slight degenerative change in the cylindrical epithelial cells. But they were very slight compared to that of gastric mucous membranous cells, and just a little stronger than those various changes which appear in physiological secreting function. This change was doubtless due to the change caused by the parenteral injection of the gastric cells, and the actions of gastrotoxin and other organ toxins.

The kidneys. This organ is affected by the parenteral injection of the emulsion of almost any organ cells or any organ toxin. This is so probably because it is something like a filter through which proteids or various kinds of toxins are excreted. To the naked eye there was a slight hypeeaemia in the cortex. But these were no macroscopically noticeable haemorrhage and necrotic patches. Under microscope, hyperaemia was strongest in the parts around the glomerulus.

In some part there was a few haemorrhagic patches. There was the effusion of a few erythrocytes in convoluted tubules. In an instance, there was no hyperaemia. In another instance, small oedema were seen pressing on the urinary tubules in various parts of interstitial substance.

There was somewhat strong hyperaemia in the blood vessels of glomerulus in some instances. Generally, besides hyperaemia, there was an increase of round cells in this part. Slight swelling and turbidity were seen in the epithelial cells of the convoluted tubules and Henle's loop. The vacuolar formation, the abnormal arrangement of granules, the degenerative process of nucleus and of the protoplasm were also observed.

By fat staining, it was found that the convoluted tubules contained somewhat large amount of fat. Especially, this was often found in over physiological quantity in Henle's loop. These changes were much slighter than those produced by the injection of kidney cells emulsion by *Kimura*. In the interstitial substance, some infiltrations of round cells were seen. I believe that the above described change

of kidneys was produced partly by the irritative action of the components of the gastric cells parenterally injected, and partly by the direct action of the absorbed parenchymatous cells of kidney which were necrotized by the primary manipulation, and partly to the action of nephrotoxin which was created in the course of time.

They all must act upon the parenchyma of kidneys. This idea agrees with the views of Bierry, Pettit and Schaeffer which they published in connection with their researches on organ toxin. They said that when various kinds of organ toxin were formed, hepatoxin and nephrotoxin almost always were simultaneously present in the immunised serum, because the various organ cells parenterally injected almost always affect the parenchymatous cells of liver and kidneys. It seems to me these words are exactly true.

The liver. Microscopically, there was often somewhat strong hyperaemia along the liver cells travecula in the liver lobules. The central vein was somewhat dilated and slightly hyperaemic. There were especially the degenerative processes in the liver cells around this part. They were fatty degeneration, vacuolar formation and the change in nucleus. There was little glycogen content in these degenerated cells. Often near the peripheral part of acinus, the pycnotic nulei which stained deeply were seen. By fat staining, a large number of fat globules of various sizes, were found not only in Kupfer's cells but also in liver cells. Their distribution was not uniform, some being found in a large number in the peripheral zone, some in the central part of acinus. But these changes were so slight as to be incomparable to the changes caused by the injection of liver emulsion. There was almost no recognizable change in the blood vessels of liver lobules, bile ducts and the interstitial substance in liver.

Suprarenal body. There was no remarkable change. No hyperaemia was seen in the cortex. The heart, There was no remarkable change except slight hyperaemia. A small quantity of fatty granules was found throughout muscle cells. The lungs. Sometimes slight hyperaemia and small haemorrhage were seen. Atelectasis was seen in many places just as in the case of acute manipulation. In the place where atelectasis appeared, there were seen many erythrocytes, leucocytes and round cells in alveolar walls. These cells were found sometimes in the pulmonary vesicles, but this kind of change was much slighter than that which was produced by acute manipulation. The spleen. It was somewhat hyperaemic and had many deposits of haemosiderin. There were erythrophagy and decay of pseudo-eosinophile cells. Lymph-gland. There was no remarkable change. The lymph-sinus was somewhat dilaed. There was very little haemosiderin in lymph follicle. Sometimes many pseudo-eosinophile cells were found in lymph-sinus. Bone marrow. There was a slight myelosis.

Section II.

The change in the membranous tissue of other part that takes place when a part of mucous membrane of stomach wall is crushed.

In order to necrotize artificially a part of the mucous membranous tissue of stomach wall, I performed laparotomy (by anesthetizing with ether) in two rabbits, and thoroughly crushed a part of the wall of the gastric body by pinching it with two fingers. Now I thought it would be interesting to examine what change will occur in the untouched part, when necrosis was induced in the tissue of mucous membrane of the crushed part. This manipulation was made to two animals and on the third day, they were subjected to autopsy. The tissue of their mucous membrane was examined. Both of these animals were more or less emaciated, and there was a decrease of the body weight by 50 gram in one, and by 100 gram in other animal. Before killing, they were lively and no change was noticed. The wound in the abdominal wall perfectly healed up and there was no change. The autopsic appearance showed no change worthy of description macroscopically and histologically except the stomach. Therefore, I shall briefly decribe the appearance of stomach only. In both of the animals, apparently there was no change in the part of stomach wall which was crushed. It was difficult to locate the part where the manipulation was applied to. When the stomach was opened, it was found that the contents were full, and in one animal a little altered blood pigment intermingled with the contents. There was much mucus in both of them. In one instance, there was a superficial erosion of the size of a thumb in the manipulated part. In other instance, there was very slight malformation of the size of a little finger which seemed to have almost healed up. Both of them, however, were covered with cells of mucous membrane. Therefore there is no doubt that the mucous membranous cells of that part was necrotized by crushing. But this action was limited to a small portion (no larger than a thumb) of mucous membranous surface, and caused no noticeable change in the local muscular coat or in other parts of mucous membrane.

I have excised a part of stomach wall which was far apart from the necrosed portion. In this way, I made the histological examination of the fundus and the pyloric glands to find the following slight changes. The change was not to be compared to that which was produced by the parenteral injection of the emulsion of the cells of gastric mucous membrane. Besides the slight hyperaemia of mucous membrane, there was more or less catarrh in the epithelial cells of mucous membrane, and on its surface, somewhat large quantity of mucus, sometimes many detrital cells, sometimes, none of them were seen. The most remarkable change in the fundus gland area was the degenerative changes of parietal cells.

The change of them were polychromasis, vacuolar formation, varying sizes, plasmolysis, and plasmoptysis. But most remarkable of all was pycnosis of nuclei. The pycnotic nuclei in the protoplasm which stained deeply interspersed here and there and displayed a remarkable appearance. There were plasmolysis, plasmoptysis, abnormal granular arrangement were seen in several parts of principal cells. A slight degenerative change was noticed in the nuclear substance. In the pyloric gland area, there was often fairly strong degenerative change in gland cells. There was almost no hyperaemia in this part. In a word, when the local necrosis is produced in the cells of gastric nucous membrane by an artificial manipulation, there will arise a degenerative change of certain degree in the cells of mucous membrane of other untouched part. But the change is very slight compared to that produced by the parenteral injection of a large quantity of the emulsion of gastric cells. I may enumerate three modes concerning the procedure of this pathological change. (1) Perhaps slight catarrh was produced by laparotomic performance with anesthetizing ether or by the direct irritation of swallowed ether. This of course is conceivable. But the autopsy was made after three days from the time of manipulation, and it is difficult to imagine how the change in the normal part could be induced in that way. (2) Performing the above described manipulation might have caused a catarrh on the surface of the nucous membranous cells. This is surely a cause worthy of consideration. (3) As the result of artificial necrosis of the tissue of mucous membrane, a portion of the components of dead cells may fall in the stomach and mingle with its content, and a portion may be resorbed by the blood and enter the circulation, and act upon the tissue of gastric mucous membrane. Also this way of resorption of the cell components is different from the resorption due to the parenteral injection of the emulsion of the gastric cells into a living body, yet the action after their entrance into the blood criculation is the same. These components in blood circulation may act upon the diseased and necrotic patch when they come round to the stomach wall, or they may act upon the normal part. This, I believe, is a mode. On account of these modes the secretion was quickened, and somewhat a large amount of mucus was present on the mucous membranous surface, and caused uniformly slight catarrhal change. Therefore slight degenerative change in the cells of gastric gland of the normal part may be due to the gastric catarrh produced there, but it may be due also to the direct action of the components of the gastric cells which were resorbed. If this were true, it is the creation of *auto-antigene* in the living body.

If the cells of gastric mucous membrane were degenerated and necrosed on account of a certain disease, a portion of these components may fall off and be lost, but other portion will enter the blood circulation and have *Miyagawa*'s so-called direct action of the components of cells upon the stomach wall.

It has already been stated that this kinds of irritation will quicken the physiological function of the cells of gastric mucous membrane. But when this

irritation is too strong, it would be natural to expect the causation of the degenerative change of cells. I believe also that the necrosis of the cells of mucous membrane and their resorption and their direct action thereby produced are constantly taking place in the normal body, and they may be regarded as a most proper irritation for the living body by which the physiological function of the cells of gastric mucous membrane is maintained physiologically in good order. I do not think that it is the only irritation by which the physiological function of the cells of gastric mucous membrane is kept going on, but it is an important one. I have already stated in the study of gastrotoxin with *Miyagawa* and others that the direct action of the components of the cells of gastric mucous membrane which are resorbed at the time of various diseases, upon the cells of stomach wall is by no means an insignificant phenomenon. I shall not discuss this subject now. But what I want to add here is the assertion that it is inevitable that the components of necrosed cells of mucous membrane of an animal which are resorbed will become autoantigen and, they, circulating with the blood would act upon other normal part of its own body. I believe the process is the same as the remarkable change of the parenchyma of the kidny of normal side produced by crushing the kidney of one side or by ligation of urether in *Kimura*'s experiment.

Section III.

The change of organs due to the injections of the emulsion of heterogeneous gastric mucous membranous cells.

I injected 2.0 gram of the gastric cells of guinea-pig into two rabbits, which were put under autopsy on the following day.

Again I made two injections of 1.0 gram of such cells every other day and then put the animals under autopsy. I examined the changes of the gastric mucous membrane and of other organs. In these two kinds of experiment, I found that the change of the organs was nearly alike to that which was observed in the preceeding experiments, and it was difficult to find out any difference.

I shall briefly describe the change in the tissue of gastric mucous membrane. There were small bleeding spots on the gastric mucous membranous surface. Haemorrhagic erosions also were noticed. The histological examination enables us to see non-haemorrhagic erosion. The change of gland cells were mainly the degenerative processes and necrosis of parietal and principal cells. The degenerative change of these two kinds of cells was not different from that which was produced by the parenteral injection of the emulsion of the homologous cells.

In the pyloric gland area also, there was no difference between this case and the case in which the homologous antigen was used.

I found that the injection of the gastric mucous membranous cells, no matter homologous or heterogeneous the antigen might be, produced the degenerative

change in the cells of gastric mucous membrane and other organs. The description concerning change of other organs shall be omitted.

Section IV.

The change of organs due to the injection of the emulsion of homologous intestinal mucous membranous cells.

(1) Acute change. (2) Chronic change.

I prepared the emulsion of the duodenal and jejunal mucous membranous cells of the rabbit according to the method which was described before. I made intraperitoneal injection of this emulsion in the following various quantities into the rabbit, and examined the change of the intestines and other organs. The experimental animals were the medium sized rabbit into which 2.0 gram was injected and which was killed on the following day, the rabbit into which 3.0 gram was injected and which was dying on the third day, the rabbit into the injection of 0.5 gram and 1.0 gram was made at an interval of two days and which was killed on the fourth day, the rabbit into which 0.5 1.0, 3.0 gram was injected every other day and which was killed on the seventh day, on the rabbit into which 4 injections of 0.5, 1.0, 1.5 and 2.0 gram were made every other day and which killed on the eighth day. Those which died were dissected immediately after death, lest the post-mortem changes may come in. Those were the cases of acute change. But in order to make the examination of chronic change, I injected 1.0 gram of the emulsion of the duodenal and jejunal cells once every week. After five manipulations, and at the end of the sixth week, I made the dissection of the animal. I shall now describe the acute and chronic changes. Clinical appearance. The general symptoms of the rabbit caused by the injection of the emulsion of intestinal cells resembled those of the rabbit caused by the emulsion of gastric cells, so it shall be omitted. Diarrhoea was often seen in them, and consequantly the decrease of their body weights during the manipulation is more remarkable.

The pathologico-anatomical appearance.

Naked eye appearance. The various changes in the peritoneal cavity of the rabbit resembled the changes seen in the case of the injection of gastric cells. There was always hyperaemia of varying degrees in peritoneum. Generally there was much mucus in the intestines and remarkable hyperaemia specially in duodenum and jejunum. In three of six instances of rabbits which had been treated with acute manipulation, there was macroscopic haemorrhage and erosion in the duodenal part. In one instance, the bleeding spots were a little smaller than the size of pin-head and their number was relatively small, counting a little over ten. In other two instances, the bleeding spots were punctiform and almost innumerable covering the whole of the mucous membrane of duodenum.

Such haemorrhagic points though small in number were found also in the

mucous membrane of jejunum. There was hyperaemia in the serous membrane of large intestine and of coecum. But this is seen generally in the case of the injection of any emulsion, and may not have any great significance. There was no remarkable change in the mucous membrane of them.

The stomach had small contents and much mucus. There was slight hyperaemia but no haemorrhagic erosion in the mucous membrane. Only in one instance, there was a bleeding spot of the size of a pinhead in the pyloric part, especially near duodenum. The changes in the liver, kidneys, and other organs were nearly the same as those in the case of the injections of the gastric cells. The main macroscopically recognizable change was hyperaemia.

Microscopic appearance.

The duodenal mucous membrane; Histologically there was almost always hyperaemia of varying degrees. Sometimes a little bleeding was noticed. In the part where the haemorrhagic erosion was discernible by the naked eye, there was of course the necrosis in the tissue of mucous membrane. It is also the matter of course that there should be non-haemorrhagic erosion. It seems that the genesis of these changes should be sought in the embolus of small blood vessels in the mucous membrane. This is possible specially because the blood vessel of intestine is the end artery. Sometimes I failed to find out this embolus. But as I said before, with regard to the genesis of the appearance, there are many reasons for believing that the necrosis of cells is the initial change. I shall not repeat it here. The change of Brunner's gland was very interesting. There was often a strong hyperaemia sometimes a little bleeding. The various kinds of the degenerative change were noticed in the gland cells. In the degenerated gland cells, the granules took very irregular arrangement and sometimes were stained very deeply. There were sometimes a remarkable vacuolar formation and plasmolysis. Corresponding to these changes, there were various kinds of degenerative processes in the nuclei. Pycnosis was specially striking. Not a few caryorhexis and caryolysis were found and often no nuclear substance was discernible in the gland cells.

In the villus of the duodenal mucous membrane, there was specially marked hyperaemia, sometimes small bleedings were seen. There were generally many round cells, and also pseudo-eosinophile cells in this part. There were also various degenerative processes in the epithelial cells of the villus. Sometimes, the necrosis of many cells of mucous membrane which were linked together was observed. If such cells undergo the action of digestive juice, they may easily become an erosion. If these necrotic patches should be pressent on somewhat extensive area, the above described nonhaemorrhagic necrotic patches will be formed. Generally there were somewhat large quantity of mucus and cell detritus on the surface of mucous membrane. By fatty staining, relatively large fatty granules were found in Brunner's gland cells. The fat of the size of a granule was found also in the goblet cells and cylindrical epithelial cells. There was a slight hyperaemia in sub-mucous layer. But almost no haemorrhage was found. There was no reco-

gnizable change in the muscular coat. I took several piecess from tne intestines without duodenum and examined them under microscope. They were upper part of small intestine, parts of jejunum and large intestine. Their change was uniformly strong hyperaemia. Sometimes the haemorrhagic erosion was recognized. I cut a portion from the part where much change was expected and made a section specimen out of it. I shall briefly describe the change appeared in it. First I must describe the change seen in Lieberkühn's gland cells. The cells were not few in which there was partial or entire lacking of cuticular margin which are physiologically present in them. In the case of somewhat strong degenerative change, there were often remarkable vacuolar formation, plasmolysis, and plasmoptysis. Not a few broken down cells were noticed. In the goblet cells, pycnosis, caryorhexis, and caryolysis of nuclear substance were not rarely noticed.

But as they take multifarious forms physiologically, we must be cautious in regarding it as the mark of degeneration. But careful examination will show that there was a degenerative change in those cells. Moreover, I felt that the number of goblet cells generally decreased in all instances. By fatty staining, I found there were no more remarkable fatty infiltration or degenerative change than appeared physiologically. There were slight hyperaemia in several parts of submucous and muscular layer. I also felt that the change of other intestinal parts was rather slight compared to that of the mucous membrane of duodenum. It is interesting to notice that in all instances of present case there was a degenerative process of higher grade compared to the change of the intestinal mucous membranous cells produced by the parenteral injection of the emulsion of gastric cells into the rabbit.

It is an indubitable fact that the intestinal mucous membranous cells parenterally injected into the living body would act upon the cells of intestines and that when the irritation is strong, the above described degenerative change will taken place.

Chronic change. I made five intraperitoneal injections of the emulsion of the mucous membranous cells of duodenum and of the upper part of small intestine at the rate of once a week. On the sixth week the animal was put under autopsy. The appearance was on the whole essentially the same as the acute change; only the change was a little slighter. A part of the mucous membrane was atrophic, but insignificant parts did not have any change whatever. A point which specially attracted my attention in this chronic manipulation was somewhat remarkable atrophy of Brunner's gland cells. There was no such remarkable change in Lieberkühn's gland. By fatty staining and the granular staining of Altmanshridde the changed cells usually become very conspicuous. But the lipoid staining of intestines must not be mixed with that which was resorbed from the intestines. Hence the amount of the contents, variation in the size of granules, should be carefuly estimated even if they were found. But most of them appeared in the inner cavity of villus. Hence, if carefully distinguished, it is not difficult to tell that which was degenerated from that which was not. From the

appearance that has been described, there seems to be no room for doubting that when the emulsion of the intestinal mucous membranous cells of the rabbit is injected into the peritoneal cavity of a rabbit, its component will be resorbed and act upon the cells of the intestinal mucous membrane.

I shall next describe the change in other organs.

The stomach. The appearance is different according as it was acutely or chronically treated. The change was slighter in the case of chronic manipulation. Generally, mucus and cell detritus were seen on the surface of mucous membrane. In gland cells, the change of the principal and parietal cells was remarkable.

The parietal cells generally stained badly, and the various degenerative changes which were seen in the case of the injection of gastric cells were seen in their protoplasm and nuclei. But these changes were slighter. The changes in the principal cells were also slighter than that caused by the injection of the emulsion of gastric cells. And there were no necrotic patch and haemorrhagic erosion. But the difference between the appearances in two cases was not the difference of quality but of quantity. Therefore I believe, if the manipulation was properly performed, we could produce necrotic patches, haemorrhagic and non-haemorrhagic erosion by injection of the emulsion of intestinal cells. By fatty staining, I could not find any remarkable change. The pyloric gland area. Mucus and cell detritus were seen on the surface of mucous membrane. There was slight degenerative process in several parts of gland cells. The kidneys. There was hyperaemia both in the chronic and acute manipulated cases. In the case of acute manipulation, there was a strong hyperaemia in the cortex and medulla. This was specially the case with the cortex, and sometimes small bleedings were seen in it. In the glomelurus there was an increase of the number of round cells, besides that of erythrocytes, and a few leucocytes were seen. A slight degenerative change in the epithelial cells of convoluted tubules was noted. Very few epithelial and nuclear casts were seen. The blood cell casts were observed here and there. Generally the main changes of kidneys were hyperaemia and small haemorrhage. The parenchymatous change was relatively slight. There was a small quantity of minute fatty granules in the epithelium of convoluted tubules. There was none in glomerulus. But somewhat remarkable fatty granules were often seen in the ascending limb of Henle's loop. They were though small in number sometimes seen in collecting tubules, and that was marked in the case of acute manipulation.

The liver. There was somewhat strong hyperaemia. Often a remarkable degenerative process was seen in the liver cells. In one instance, there were necrotic patches, in the peripheral zone of lobules. In the place where was no necrosis, the degenerative change of liver cells was obvious. In other instances, various degenerative processes of liver cells were alse seen, but they were not so serious. In the case of chronic manipulation hyperaemia and the change of liver cells were slighter. By fatty staining, relatively a large quantity of fatty granules

was sometimes noted in the liver cells.

The lungs. Generally they were hyperaemic and had small bleedings in several parts. Sometimes the capillary blood vessels were filled with hyaline mass. Many erythrocytes, leucocytes and round cells were present in the alveolar walls. Sometimes alveolar cavity seemed to be hard pressed by them. In such a part, many pseudo-eosinophile cells were seen in the alveolar wall. These cells were sometimes seen in the alveoli, although their number was very few.

The heart. The heart was somewhat hyperaemic, and fatty granules were seen in several parts.

The suprarenal body. The change was not much different from that produced by injection of the emulsion of gastric cells, and its description will be omitted.

The spleen. It was strongly hyperaemic and the spleen sinus dilated. There were little deposit of haemosiderin and the phenomenon of erythrophagy was very rare. There was no decay of leucocytes in follicles.

The bone marrow. There was slight hyperaemia. Sometimes myelosis was very remarkable and sometimes not. Generally, the change was the same as that seen in the case of injection of emulsion of gastric cells.

Chapter V.

Summary and conclusions.

By summarizing the experimental appearances that have been described thus far, the following conclusions may be drawn. A given amount of the gastric mucous membranous cells of a rabbit or a guinea-pig was well ground in a mortar and physiological salt solution emulsion or autolysate by sterilised distilled water were made out of it. This was injected into the peritoneal cavity of the rabbit which was killed and put under autopsy at various periods. The action of the injected cells upon the tissue of the gastric and intestinal glands and other organs were examined. When the quantity of injection is somewhat great, or from 3.0 to 4.0 gram of the gastric or intestinal mucous membranous cells is injected into the rabbit weighting about 2000 gram, the animal will show violent symptoms and often be found dying on the following day. But if the quantity is reduced, the appearance of symptom would of course be slow ; but it would be very severe. If about 1.0 gram is applied, no remarkable change will occur and immunization could be repeated for a long time.

If the homologous or heterogeneous gastric cells emulsion is injected at the rate of 1.0 gram per kilo, slight erythrocytemia and remarkable leucocytosis will apear. If the injections are made repeatedly, these erythrocytemia and leucocytosis will become more and more remarkable. In no instance leucopenia is seen. Leucocytosis begins in two to four hours after injection, reaches its top in six, eight or ten hours, and after two to four days, the normal state takes the turn. Leucocytosis is mainly due to the increase of pseudo-eosinophile cells. When the

emulsion of the gastric or intestinal cells is parenterally injected, no matter homologous or heterogeneous the cells may be, they cause changes respectively to the gastric or intestinal mucous membranous cells. In order to make the change very conspicuous, sometimes I used a large quantity. But at other times, I used relatively small quantity in order to examine the chronic change which appear in the process of long duration. But acute or chronic, the change produced in the cells of tissue is essentially the same.

They were all degenerative change, necrosis, erosion or ulceration and sometimes atrophy of gland cells. In case of chronic manipulation, hypertrophy of interstitial substance may follow the atrophy of the tissue of mucous membrane.

This is the same as the atrophy of kidney parenchym which *Kimura* produced by using kidney cells emulsion. The hypertrophy of interstitial substance can not be ascribed to the direct action of cell component only, for there is no doubt but the chronic manipulation could accelerate the formation of gastrotoxin or enterotoxin which will all act upon the cell in producing the said effect. Doubtless, parenterally injected component of cells of mucous membrane which enters the blood circulation would act upon the survived cells of mucous membrane. In order to magnify this action I purposely used a large dose, and consequently the degenerative process and necrosis have taken place. If the quantity of injected cells is small and its irritation is kept within physiological threshold, the cells of mucous membrane will quicken their physiological function by this irritation.

Such a phenomenon may be always present in the physiological process of living body, as cells of mucous membrane are constantly died out and a portion of their component will pass into the blood circulation. Suppose, for instance, in the cases of peptic ulcer, cancer or an acute datarrh of digestive tract which are produced by various causes, extremely large quantity of the component of dead cells is absorbed into the blood circulation. It is not difficult to imagine that its irritation will be more than physiological threshold and may give rise to the direct action and bring a powerful action upon the diseased or other normal parts. The violent diarrhoea seen in dysentery, and cholera, may be due to a severe catarrh on the surface of mucous membrane caused by the bacilli or their toxin, but we must also consider the uniformly and strongly acting direct action of the component of cells which are necrotized by these diseases and which entered the blood circulation.

Of the changes of gastric mucous membrane produced by the injection of the emulsion of gastric cells, the most interesting is the change in the parietal cells. These cells may have weaker resistance than the pricipal or the pyloric gland cells.

Again we can recognize a remarkable degenerative process in Brunner's gland by the injection of the emulsion of duodenal cells. These are the interesting subjects for further investigation.

A few words about erosion and ulceration produced on the gastric and

intestinal mucous membrane may not be out of place.

As the result of above described manipulations, severe hyperaemia, often haemorrhage on the surface of mucous membrane, which would bring the necrosis of tissue, or in other words, haemorrhagic erosion may appear. But when non-haemorrhagic erosion is examined it is found that there is the embolus in the small blood vessel on the basal part of mucous membrane. It may be that the tissue of that part is necrotized on account of such embolus and subsequently digested and eroded. Or it may be that the cells in certain part are primarily necrotized by a strong action of the resorbed component of the cells of mucous membrane. When these histological appearance is taken up together with the genesis of necrotic patch in other organs by the use of the emulsion of the same organs, we may affirm the possibility of the formation of such erosion. The change in the tissue of the gastric or intestinal mucous membrane produced by the parenteral injection of the cell emulsion of liver, kidney, supra-renal body, lung and testicle into the living body is always slight, and the difference is remarkable compared to the change due to the direct action caused by the use of the emulsion of homologous cells. What has been stated is a summary of the action of the cell component of an organ upon the homologous cells. But when the cell component of mucous membrane of digestive tract enters the blood circulation, it would act not only upon the tissue of the mucous membrane of the digestive tract, but almost always upon the kidneys, liver, and haematopoetic organs, producing various changes there. Hyperaemia, haemorrhage or necrosis are found in kidney and liver. This is an interesting fact, and may have great significance when it is considered in connection with various clinical diseases. But this chage is slight compared to that produced in those organs by the same amount of kidney or liver cells emulsion.

Conclusions.

(1) When a certain amount of autolysate or emulsion of mucous membranous cells of digestive tract of the rabbit or guinea-pig, is injected pareaterally into a rabbit, various kinds of degenerative changes and necrosis, hyperaemia, haemorrhage, often the haemorrhagic and non-haemorrhagic erosions and ulcer will appear on the gastric mucous membrane of the injected rabbit. Those changes are produced by the direct action of resorbed cell components as Dr. *Miyagawa* stated.

(2) When such manipulations are continuously made, there will be final atrophy of the gland cells of the mucous membrane and hypertrophy of interstitial substance.

(3) When a certain amount of the emulsion of the mucous membranous cells of duodenum and small intestine is injected into the peritoneal cavity of the rabbit, degenerative changes and necrosis, hyperaemia, haemorrhage, sometimes haemorrhagic and non-haemorrhagic erosion and ulcer will occur in the intestinal mucous membrane of the injected rabitt.

(4) When the emulsion of kidney, liver and other organs is parenterally injected into the living body, the above described change in the cells of gastric and intestinal mucous membrane will be very slight.

(5) When the emulsion of the mucous membranous cells of digestive tract is parenterally injected into the living body, various degenerative changes will happen in liver, kidneys and other organs. But the change is slighter compared to that produced by the injection of the same quantity of the emulsion of the same organs.

(6) Blood picture. When the emulsion of gastric mucous membrane, irrespective of homologous or heterogeneous, is parenterally injected, there will be slight decrease of erythrocytes and strong leukocytosis. Leukocytosis is due mainly to the increase of pseudo-eosinophile cells.

Many thanks are due to Dr. *Nagayo* who gave me many valuable suggestions, to Dr. *Miyagawa* who has guided me in all this research work.

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Description of Plates.

Fig. 1. Section of a rabbit's stomach from a case in which the animal received three successive injections of the emulsion of mucous membranous cells of the stomach of a rabbit and was killed on the fourth day. There are such remarkable changes as polychromasis, variation of sizes, pycnosis and decay of nucleus in the parietal cells of fundus gland. Degenerative changes in principal cells. Slight hyperaemia.

Fig. 2. Section of a rabbit's stomach from the same case as Fig. 1. Formation of hyaline embolus in capillary blood vessels in the tissue of gastric mucous membrane. Owing to this change, the tissue of gastric mucous membrane supplied by these blood vessels formed non-haemorrhagic necrotic patch.

Fig. 3. Section of a rabbit's stomach showing atrophy of gland cells and consequent secondary increase of connective tissue caused by injection of the emulsion of the rabbit's gastric mucous membranous cells into the rabbit once a week.

Fig. 4. and Fig. 5. Sections of a rabbit's stomach from a case of my subacute manipulation in which 4 injections were made every other day, and the animal was killed on the 8th day. Fig. 4. Shows haemorrhagic, Fig. 5. non-hremorrhagic erosion.

Fig. 1.

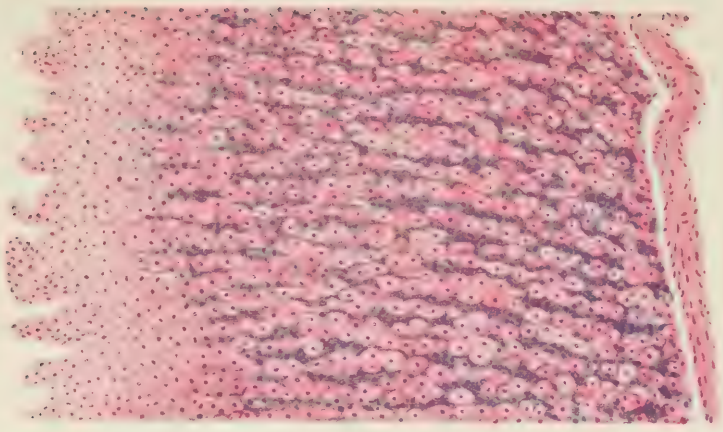


Fig. 2.

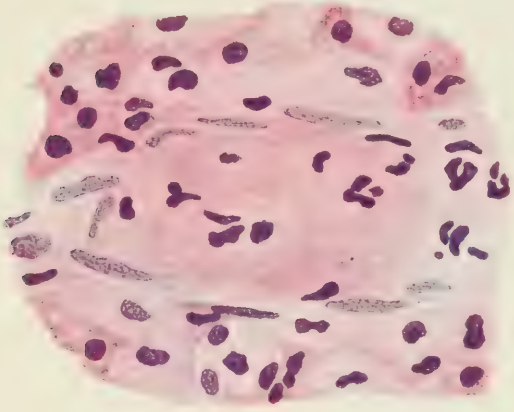
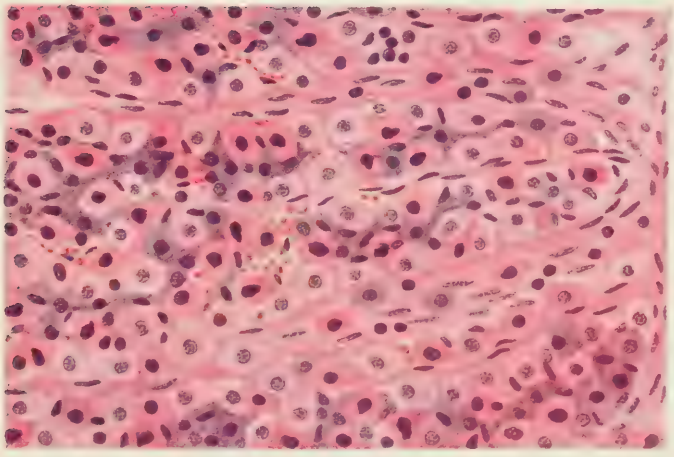


Fig. 3.

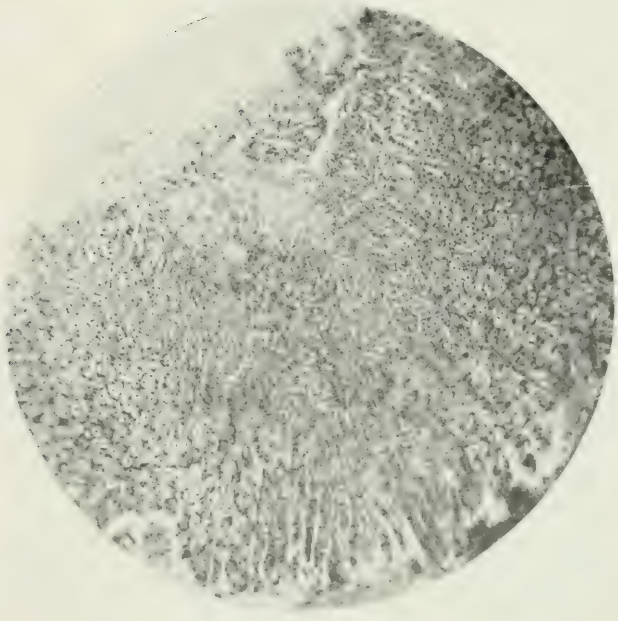


(Dr. H. Murai : The Influence of the Mucous Membranous cells of the Digestive Tract Parenterally Injected on the Organ on Tessue.)

Fig. 4.



Fig. 5.



(Dr. H. Murai : The Influence of the Mucous Membranous cells of the Digestive Tract parenterally Injected or the Organ or Tissue.)

A Study on Kidney Cells as Toxin or irritative Substance and its Relation to the Promoting of Nephritis.

By

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Contents.

I. Introduction.
II. The experimental materials and the methods of investigation.
III. The pathological findings created by the emulsion of kidney cells which was injected parenterally, upon kidneys or other viscera (The direct action of resorbed components of cells upon the kidney parenchyma).
  1. The case of injecting of the emulsion of homologous kidney cells.
  2. The case of injecting of the emulsion of heterologous kidney cells.
  3. Changes of kidneys or other viscera induced by repeating injection of the emulsion of homologous kidney cells for a long time (the formation of chronic interstitial nephritis).
IV. The pathological findings of kidneys in the case of crushing a part of a kidney or binding a ureter on one side.
  1. The case of crushing a kidney on one side.
  2. The case of binding a ureter on one side.
V. Experiments on nephrotoxin.
  1. The case of injection of nephrotoxin under the fibrous capsule of a kidney on one side (the action of isonephrotoxin).
  2. The case of injection of auto-immune (its own) serum under the fibrous capsule of a kidney on one side of experimental animal which showed the formation of isonephrotoxin (the action of autonephrotoxin).

VI. Summary.
VII. Conclusion.

I. Introduction.

When there is the degeneration or the necrosis in marked degree in kidney epitheliums produced by primary or secondary nephritis or other various causes, a part of the component of the epithelial cells is excreted into urine being shed into the tubules and another part is resorbed into circulation. Though in such a case the action of this resorbed component of the epitheliums may have a very important significance, it seems to be still an unsolved problem.

According to Dr. *Miyagawa*'s advocacy; "If the tissue or organ cells are parenterally injected into the organism, they act upon the homologous tissue or organ-cells. The organtoxin responsed to the injected cells is generally created in the organism," such resorbed part circulating the blood vessel is to act upon the healthy parts of the kidney. Intending to solve this problem under the direction of Dr. *Miyagawa* I have exactly inspected the changes in kidneys and other viscera after parenteral injections of homo- and heterologous kidney cells emulsion to rabbit, and recognized some intense changes in the kidney on one side when the definite lesion being induced by the artificial process in the kidney on the other side. And then forming nephrotoxin by repeated parenteral injection of the emulsion of kidney cells, I found the chronic change of kidneys in such occasion, that is the nephritis interstitialis. After this, using nephrotoxin and producing cytolysis in a kidney on one side I have inspected the changes on the other healthy side. From these experiments above mentioned it became evident that the parenteral resorption of kidney epitheliums resulted in Dr. *Miyagawa*'s so-colled direct action upon the kidney on the other side and produced especially the remarkable pathological changes to the kidney, so it became possible to compare these pathological changes with such as produced by nephrotoxin. Above statement is chiefly from the histological stand point, but moreover I inspected the existence of albuminuria in order to know from another point of view, that is from the function of the kidney. Up to this present time we have numerous literatures dealing with the works about nephrotoxin. But many of them confuse the effects of the direct action with those of immune body. That is the reason why I especially studied distinguishing both these actions. By above mentioned experiments I found that the both actions — the direct action of the resorbed kidney parenchyma and the action of nephrotoxin — were never to be neglected, and confirmed the influence of the pathological changes on one side upon parenchymal cells on the other side, and then illustrated that both actions had important significance to creation and to forthcoming of the desease.

Abstracts of literatures dealing with this point, are as follows.

On the contrary to the numerous literatures dealing with nephrotoxin, there are very few which stating about the direct action above mentioned. Herzen[27] studied concerning the resorption when the emulsion of kidney cells was intraperitoneally injected into an animal and reported that the degeneration took place in kidney and liver. In his statement, he did not differentiate the so-colled direct action of resorbed cell component and the nephrotoxin produced by immunization. Albarran and Bernard[1] found the parenchymatous degeneration in the liver and kidney of the rabbit perished by the subcutaneous injection of kidney cells emulsion of guinea-pig, and found also the same degeneration in the case of the injection of liver emulsion. So they asserted that it was impossible to recognized the organ specificity of nephrotoxin. But I presume that their findings on animal was created by the direct action because of its rapid death. Suñer found that if both kidneys were incised, the blood of this animal became toxic to the kidneys after lapse of 24–36 hours when it was injected into other healthy animal, being decreased in the quantity of urine and containing albumen in urine. On the contrary, directly after the kidney operation, the blood was obtained and injected into another animal, being increased in the quantity of urine. Kapsenberg[3] recognized the above results as the action of nephrotoxin, but I presume that this is the direct action of broken materials of kidney parenchyma upon living part which entered into circulations. Furthermore Kapsenberg found necrosis in non-operated kidney parenchyma in one side of the guinea-pig which died two days after had been tied the kidney stem in the other side. And he attributed it to the formation of autonephrotoxin. But I consider this the result of the direct action. Lindemann,[4] the first researcher on nephrotoxin, experimented that the immune serum of guinea-pig, injected with the emulsion of kidney cells of rabbits, was inoculated intravenously into rabbits which were suffered from albuminuria and uraemia ; and he recognized histologically the degeneration of tubules epitheliums. These changes were not to be recognized if normal serum was employed. In another experiment he observed the formation of nephrotoxin in dogs which were afflicted with nephritis by chromic acid. Namely when the serum was injected into other dogs the effect was the same as in the former experiment, and resulted in acute hemorrhagic nephritis. And he asserted, the toxity of the serum was propotionate to the degree of changes of kidney parenchyma of the animal which supplied serum. Afterwards many works appeared in rapid succession recognizing the existence of nephrotoxin. Bierry[6] observed changes of a kidney on one side by means of binding the artery of a kidney on the other side, and affirmed the existence of autonephrotoxin. Hulot and Romont[7] reported the same case as Lindemann.

Ascoli and Figari[8] examined heteronephrotoxin which was obtained from rabbits, and attempted from this fact to illustrate the producing of chronic nephritis. Anzilotti[9] observed hyperaemia of glomerulus and turbid swelling in tubules uriniferus on one side when he bound ureter or artery on the other side of a rabbit ; and he attributed this to autonephrotoxin. Play und Corpechot[10] found the increase of connective tissue and partial disappearance of epitheliums in kidneys of animals which were injected nephrolysin, and as this toxic action was carried to embryo, he intended to illustrate it as a cause of the hereditary nephritis. Bierry and Meyer[11] observed that nephrotoxin which was obtained by immunization with nucleoproteid from the kidney parenchyma instead of the whole cell, occurred intense albuminuria in animals injected with this immune serum. But this phenomenon did not showed the specificity to the kidney. In comparing the effects of nephro- and hepato-toxin Sata[12] asserted that though such cytotoxins caused the same changes acting upon homo- and heterologous animals, the action upon the homologous was more intense, especially more rapid

to homologous organ and later to the other viscera. Namely the injection of nephrotoxin affected at first the kidneys, and then the liver after some time. On the other nand he observed that the injection of nephrotoxin into kidney parenchyma caused hemorrhage, necrosis and round cell infiltration in the same kidney, but no changes in liver. Moreover he recognized the specificity of immune serum by the method of selective adsorption which was employed on this line. Thus he concluded that nephrotoxin and hepatotoxin had specificity in quantity but not in quality. Castaigne[13] observed a certain change of the kidney on one side after crushing the other. Bierry, Pettit and Shaeffer[14] considered that the formation of cytotoxin was one of the effects of the damage of kidneys and liver of immunized animals, and they were agreed with that the immune serum obtained by immunization with nucleoproteid of viscera had organ specificity and therefore the immune serum always affected respectively liver and kidneys. They found moreover that the formation of nephrotoxin was only seen in such a case in which the function of the kidney in one side was interrupted and the other side showed pathological changes. Néfédieff[15] experienced that the immune serum of rabbit which was obtained by subcutaneous injection of the emulsion of kidney cells of guinea-pig, induced hyperaemia and parenchymatous degeneration in the kidneys; and examined that, after its injection into other rabbits, the serum of rabbit of which the ureter on one side being bound showed the same symptom above mentioned, and was more strongly in its action at the lapse of 4 weeks than 3 after the operation. Cawadias[16] made an experiment that the serum of the patient suffering from uraemia had more than double in intensity to kill guinea-pig than the normal serum. And as both serum showed the same pathological changes in injected guinea-pigs, he concidered that there was the same toxic substance, having only a different intensity in their action The increase of toxity was not seen when there was no spasm in patients to be observed. Halpern[17] found the generation of complement fixing body in animal by injecting the cells emulsion of its own kidney which was exstirpated, as antigen, the body showed organ specificity though often fixed with emulsion of cells of other viscera and sometimes showed the species specificity. Kapsenberg[3] recognized always some changes on one side of the kidney when he bound a ureter or a kidney stem on the other side. The albuminuria which appeared on this occasion continued for a long time, and became intensive with a certain lapse of time after the operation. Under such studies he asserted the formation of autonephrotoxin. He found also the appearance of albuminuria after the injection of serum of the animal, which were operated as above mentioned, into the vein of another animal. And he intended to prove the existence of isonephrotoxin from this investigation. Lüdke, Heerman, Schüller and Leonard[18] found formation of auto-, iso- and hetero-nephrotoxin by injection of emulsion of kidney cells into an animal and also by resorption of the component of damaged kidney cells. But there was no absolute specificity, and the serological experiments were also ended without result. Wilson and Oliver[19] repeated Pearce's work,[21] and found characteristic histological change in glomerlus and slight degeneration in tubules. And judging from the experimental result of selective absorption about the immune serum, they considered they might be able io assert that nephrotoxin had the specific affinity to kidney.

All work above mentioned adovocate the existence of nephrotoxin. But on the contrary there are also some research works which are against to the above mentioned opinion, which are made by Albarran and Bernard[1] etc.

According to the works descrived above we classify nephrotoxin in three species, namely auto-, iso- and hetero-nephrotoxin. And there are many methods to examine its formation and its existence. The first method is to examine by

the disturbance of the function of kidney, the second by the pathological histological changes, and the third by the serological standpoint.

In my experiments, first, I intended to decide the meaning of the direct action of kidney cells emulsion with regard to the origination of nephritis, which Dr. *Miyagawa* asserted strenuously and second, we have already many experiments which were made by binding a ureter or crushing a portion of a kidney on one side, but all these were made only for testifying autonephrotoxin. Hereupon I examined these experiments setting my mind always on the direct action upon the other non-operated side. Third, though there were many methods to examine nephrotoxin, but no one made the injection of immune serum under the fibrous capsule of a kidney on one side in order to see the results on both kidneys. These are the reason of my attempt to examine the important meaning of the direct action of kidney cells emulsion, and to suplement something to the experiments on nephrotoxin.

II. The experimental materials and the methods of investigation.

The experimental animals were middle-sized rabbits. The emulsion of kidney cells was obtained from the kidney of rabbits from which blood was previously eliminated as complete as possible by infusing physiological saline solution into the thoracic aorte, grounding up it well, making emulsion in physiological saline solution and then filtering the emulsion with wire-net. Then I injected it intraperitoneally into other normal rabbits. I never used this emulsion after the lapse of more than one day after prepared. These procedures were of course carried out aseptically.

Albuminuria was examined every time by urine obtained by catheterization, and all examples were compared before and after the experiment. When I was unable to get urine by catheterization on account of the empty bladder, I repeated it two or three times a day. In such cases it is most important to be careful about uretheritis and cystitis. *Kapsenberg* was so careful that he made a special cage to get urine, but my experience proved it useless, if strict disinfectant precaution was performed. Albumin reaction was carried out by the supper-natant fluid of urine which was obtained by centrifugalisation.

III. The pathological findings created by the emulsion of kidney cells which was injected parenterally, upon kidneys or other viscera (The direct action of resorbed cell component upon the kidney parenchyma).

Prior to the study on nephrotoxin I injected into rabbits with the emulsion of iso- and hetero-kidney cells interaperitoneally, which was obtained by the method

mentioned above. Thus I intended to inspect precisely the direct action of the emulsion of kidney cells upon viscera, especially upon kidney, and moreover its relation to the formation of nephritis.

1. The case of injection of the emulsion of homologous kidney cells.

This experiment was performed with four rabbits and resulted in the same effect. The daily dose injected was 1.5 gram of the kidney per kilo of the body weight. All of the rabbits were autopsied on the third day after second injection. A few minutes after the injection, all rabbits lied on its belly in a corner of the room streching its hind legs and showed hasty respiration and palpitation. Then though it became able to walk after about thirty minutes it was still not lively and inactive in mode. On the next day yet it did not revive and had no desire for food. The symptom after the second injection was almost the same as above mentioned but more remarkable. Thus one animal died in the early morning on the third day and the other three, exhausted all their physical strength at the same time, became impossible to walk and were at the point of death and offered no resistance under the vivisection. All decreased very much in weight, and two of them had an attack of diarrhoea.

As to albuminuria *Herzen*[24] found its appearance from the next day after intraperitoneal injection of the emulsion of kidney cells. *Kapsenberg* also observed it by intravenous injection of the emulsion of kidney cells. *Linossier* and *Lemoine*[25] experienced always the same phenomenon when they injected parenterally heterologous albumen, and they asserted the existence of toxic substance to kidney in it. The urine of all rabbits used in my experiment showed weak alkaline reaction and no albumen before the injection, while became neutral or weak sour on the next day after injection and showed albumen obviously.

At autopsia the peritoneum was congested universally. It was more remarkable in omentum majus, on intestinal canal especially on vermiform process; and whitish yellow fibrous masses were depoted in verious places, especially distinct on the intestinal wall, though they were able to loose easily each other. Examing these masses microscopically it was observsd that it consited of cell detritus and infiltration of many cells in it. According to *Herzen*'s study, the emulsion of homologous kidney cells was resorbed more slowly than the heterologous, and the masses which were observed in this experiment were perhaps the rest of the emulsion of kidney cells, and it coincided with *Herzen*'s experience that there was never to be seen such in the case of injection of the emulsion of heterologous kidney cells.

The pathological findings of the kidneys were almost the same in both sides,—that is, slight swelling, the capsule easily stripped off and the surface of the kidney smooth. The cut surface was opaque, turbid, showing marked hyperaemia. Blood vessels dilated along the direction of straight tubules, and

even pin-head hemorrhages which were visible macroscopically were dotted in the cortex and the medulla.

Micorscopically there were also remarkable hyperaemia and the blood capillaries between tubules swelled up especially at the ramifying point of star veins and at the bordering portion of the cortex and the medulla. Hemorrhages were seen in dotted form in places of the cortex and the medulla and the parts of kidney parenchyma in adjoining of the remarkable hemorrhage were compressed. The glomeruli were also full of red blood corpuscles showing obviously hyperaemia and consequently strongly swelled having no gap between Bowmann's capsules. Hemorrhage was rarely to be seen and there was no cell infiltration which was to be regarded as glomerulitis. The nuclei of the endo- and epi-theliums in the glomerular tufts showed often pycnose. In the epitheliums of Bowmann's capsules we found also the irregular distribution of protoplasm but there was no desquamation of cell. In some examples large fat granules were percieved in a few number of glomeruli, and a few small fat granules in epitheliums of Bowman's capsules. These changes were funther slight than those of sinuous and straight tubuli. The part which underwent intense changes in sinuous tubuli, was in the cortex near the medulla, and showed acute degeneration. The epitheliums were degenerated and swelled up in places or took irregular forms, and the arrangement of protoplasm granules was very irregular, such as in one side was aggregated and in the other side was thin, or was thick arround the nucleus. Vacuoles were to be found in protoplasm here and there, namely the plasmolysis and plasmoptysis were evident. In nuclei there were also many regressive metamorphosis. We observed pycnosis, vacuolar formation and the decline staining property etc. and some of them dropped into the lumina becoming nacked nuclei, or some of them stripped off with degenerated protoplasm. In the part desolated intensively only one degenerated nucleus was seen in a transsection of a tubulus, all other nuclei being off and noly degenerated protoplasm existing in irregular form. Or nuclei were disappeared entirely and necrotized in homologous mass, or all epitheliums were stripped away and remained only membrana propria. In these lumina existed albumen masses in various sizes. Such changes were mostly to be seen in the transitional part to medulla, but also in the various part of the cortex. Fatty degeneration was also to be found in places of sinuous tubuli. But in comparing with those of Henle's loops it was more rarely and generally in slight degree. And the degree of fatty degeneration was not all the same in all urinary tubules, and in some there existed almost no fat. In straight tubules there was remarkable degeneration in Henle's loops, but in comparing with that of sinuous tubules it was somewhat in slight degree and the normal cells existed between the degenerated ones. But in degenerated epitheliums there were vacuolar fromation, plasmolysis and plasmoptysis in protoplasm as in sinuous tubules. In nuclei we found pycnosis, decline of staining property, vacuolar formation and sometimes slough of cells. These changes were remarkable in ascending shank. Conspicuous fatty degeneration

was observed also in the epitheliums of Henle's loops. Fat globules were various in sizes, many of them were comparatively large and existed mainly in periphery of cells and remarkable parts of it were deposited with fatty globules. The fatty degeneration was to be seen in all parts of the loops of Henle but the grade was different in every canale and it was intense in ascending shank. Some examples did not show obvious increase of fat and slightly beyond the physiological limit. Besides these changes in the lumina hyline and granular casts and stripped epitheliums ranged in small numbers here. Some formed epithel casts, some became so-called nucleus casts by stripped nuclei and they were dotted in places, or sometimes all these nuclei were broken. Among these various findings it was impossible to find out the cast made from blood corpuscles. These many casts existed of course also in collective tube. There were almost no other pathological changes in epitheliums of collective tubes without the fat, granules which were dotted generally in some rabbits. In interstitial connective tissue of the cortex and the medulla there were dotted slight oedema besides hyperaemia and hemorrhage in every part, but no cell infiltration. To summarize above mentioned changes they were obstacle of circulations and acute parenchymatous degenerations of sinuous tubules and epitheliums of the loops of Henle.

In liver and suprarenal glands it was observed slight parenchymatous degenerations and the other viscera were also examined but showed no remarkable changes.

2. The case of injecting of the emulsion of heterologous kidney cells.

I injected intraperitoneally into rabbits with kidney cells of ox and horse as emulsion 0.75 and 1.5 gram per kilo of body weight. Though we have various opinions as to the strength of the reaction by injections of hetero- and iso-organ emulsion, in my experiments the former showed the same but slighter symptoms as the latter and there were neither diarrhoea and nor the decrease of weight. In every example albuminuria was found but not so intensive as in the case of injection of isoemulsion, and it was very hard to prove its existence distinctly on the next day after the injection.

The general pathological findings were almost the same as in the case of injection of isoemulsion (Fig. 1 & 2). So I will state only a few important points of difference. The increase of peritoneal fluid and sediment of fibrous mass which were observed in the case of injecting of isoemulsion were not to be found in the peritoneal cavity. In the pathological findings of kidneys also there were principally degenerations in epitheliums of tubules and no remarkable difference to be mentioned. The only point as the difference was as follows. There was to be observed a few round cell infiltration between the tubules. As this infiltration existed especially in the neighborhood of the extremly destroyed tubules, it was supposed to have generated secondarily after parenchymatous lesion. If this imagination might be

correct, the chronic change would be performed gradually by repeated injection of kidney cells.

In other viscera exept kidneys there were no remarkable point to be mentioned.

Though the direct action of the emulsion of iso- and hetero-kidney cells injected parenterally upon the kidney is obvious according to these experimental results, we must take the following fact into consideration. As various substances which are injected parenterally into an animal also pathological changes in kidneys produce in a certain degree, when they are excreted from kidneys, so it is somewhat premature to assume all degenerations in kidneys as the direct action. But I was convinced of the importance of the direct action by various investigation about it in our laboratory on mucous membranes of stomach and intestine, liver, lung, testicule, lymphocyte[32] [26] etc. and *Naswitis*'s[38] and *Furukawa*'s recent studies[28] on red blood corpuscle. And in the above various studies in our laboratory the findings of kidneys were not definite in every time and the degree of degeneration was very slight in comparing with my experiments. These were the point of obvious difference of pathological findings between their experiments and mine, and they were the reason of my decision to assert the changes in my experiments as the direct action of the emulsion of kidney cells injected parenterally.

The following task was to examine their relations with the generation of nephritis. Suppose here nephritis generated by primary or secondary causes. A component of degenerated cells of kidney parenchyma will enter into circulations and influence the direct action upon other healthy parts as if kidney cells emulsion injected parenterally will do. And when the quantity is little it strengthens the functions of kidneys and strives to compensate them, but when the quantity is more and the irritation is too intensive, then the functions of kidneys will be checked and then their degeneration will appear in parenchyma. My experiments showed the latter symptom artificially. Thus there may be appear circulus vitiosus acting directly upon others entering into circulations after degenerated by the direct action, and may produce more complex relations forming nephrotoxin after a certain lapse of time. The process of nephritis is clinically easily to become chronic and the difficulty in its healing may be caused by the reason as above mentioned. It seems to me full of interest that in physiological state a certain number of kidney cells are daily undergoing to death and a certain quantity of the dead cells components is entering into circulations stimulating other normal parts and consequently quickening and regulating their function, that is to say autoregulation, without control of the nervous system. I can not help admiring the wonders of nature.

3. Changes of kidneys or other viscera induced by repeating injection of the emulsion of homologous kidney cells for a long time (The formation of chronic interstitial nephritis).

The injected kidney parenchyma was about 1.0 gram per kilo of body weight and as emulsion was injected intraperitoneally once a week and the animal

autopsied after 5, 6 or 9 injections at the lapse of one week after the last one.

Pathological findings in this line differed from those in the first experiment, almost no hyperaemia being to be found in whole kidney. In endotheliums and epitheliums of the glomerulus, hyperplasia of connective tissue was to be observed besides the obvious pycnosis of nuclei. In its remarkably changed parts whole glomeruli were changed into connective tissue as to be seen in granular kidney. And the connective tissue was combined with that of wrapping sinuous tubules near by.

The changes in sinuous tubules were almost the same as stated in the first, especially they were remarkable in the deep part of the cortex and were also dotted. Albumen masses were to be found in places of the lumina but epitheliums were few stripped off, and there was no image to obstruct the lumina as in the direct action. Here also the increase of connective tissue was to be observed, and the lapse of more than two months during nine times injections made it more remarkable than that of one month during five times injections. In medulla also it was found the increase of connective tissue where the loops of Henle was intensively disolated, and slight round cell infiltration. Namely there existed evidently nephritis interstitialis. This phenomenon was to be so explained that kidney parenchyma atrophied gradually and the connective tissue took the place of it. Thus it was possible to suppose that successive injections would cause secondary granular kidney. Considering this generating mode which I have stated already in the first Paragraph of the third Chapter, kidney parenchyma suffered from irritation by parenteral injections of the emulsion of kidney cells, and generated degeneration and at last necrosis when the irritation was too intensive. The repetition of such procedure at the definite interval produced immune body in the injected animals besides above mentioned pathological changes, and this immune body undoubtedly acted upon its own kidney parenchyma by mediation of blood circulation. All as follows inform us on these matters,— *Bolton*'s and *Miyagawa*'s[31] studies on gastrotoxin, *Noda*'s[36] and *Fujimoto*'s[33] investigations on liver, *T. Nakamura*'s[34] work on sympathetic ophthalmia and *H. Nakamura*'s[35] reserch on testicles.

In the other viscera there were no important changes to be mentioned but only slight degenerations comparing with those of kidney.

IV. The pathological findings of kidneys in the case of crushing a part of a kidney or binding a ureter on one side.

Many experiments made by others which used the same method as mine were all performed only for examining the formation of nephrotoxin but not to research the direct action upon the other non-operated side which was acted by the kidney parenchyma entering into blood circulations by the damage or necrosis of the kidney in one operated side. This was the reason why I repeated the same experiments again. Thus I wanted to examine the pathological changes in

the other kidney produced by the direct action and the formation of autonephrotoxin.

1. The case of crushing a kidney on one side.

The laparotomy was carried out always under the ether narcosis, and a part of a kidney was crushed between the forefinger and the thumb. In these occasions the animals showed symptoms like shock, but were easily revived by artificial respiration. The one fell dead on the third day decreasing in the body weight, however, the other was still full of vigour after the lapse of thirteen days decreasing little in the body weight. Then I autopsied it.

Pathological findins of the operated side : — The former rabbit showed yet hemorrhage under the fibrous capsule of the crushed kidney and hyperaemia was generally remarkable but in the latter the wound scared already and the border of the cortex and the medulla at the lesion became indistinct. No hyperaemia existed. I will give a simple summary of microscopical findings. There were in places macroscopic necrotic patches both in the cortex and in the medulla, having infiltration of round cells. In another parts also there were severe changes.

Occurred by the direct action of the resorbed cell component of the operated kidney, I found a characteristic in both above examples of intensive hyperaemia comparing with the crushed one. Microscopically there was also hyperaemia especially more remarkable in the bordering part of the cortex and the medulla. In some glomeruli were remarkable in hyperaemia and I could percieve a little hemorrhage by exact research and more intense hyperaemia in parts connecting with them. Interstitial capillaries were full of red blood corpuscles and were overflowed also in the lumina of tubules. The degree of hyperaemia in the kidney of the rabbit which was autopsied on the thirteenth day after the operation was slighter than that in which fell dead on the third day after the operation. I thought this was caused by the lapse of a fortnight. In glomeruli there were generally numerous cells and slight infiltration of round cells and a few vacuolar formations were also in the epitheliums of Bowmann's capsule. The sinuous tubules especially near the confine of the cortex and the medulla had vacuoles in various sizes in their protoplasm, and brush border vanished. The distribution of granules was very irregular and showed plasmolysis and plasmoptysis. The desquamation of cells was to be found but not so remarkable. In nuclei I perceived increase or decrease of staining degree and rarely the lumina were obstructed by gathering degenerated nuclei. But the greater part of the cortex showed no change in spite of degenerations in kidney epitheliums as above described. As to the straight tubules some changes were to be found especially in the loops of Henle. The parts affected intensively showed necrosis of epitheliums and only basal membrane surrounded albumen lump. Such degenerated parts were not universal but scattered, and it was very interesting to see that some healthy canales existed among degenerated ones, that is as if whole degenerated canales would appear being influenced by the passage of a toxin. Interstitial connective tissue also proliferated

in the kidney of rabbit, which lived two weeks after the operation, and in this part there was round cell infiltration showing the appearance of the nephritis interstitialis in slight degree. It was full of interest to compare it with the pathological changes of the kidney which was brought about by parenteral injections of the emulsion of kidney cells once a week (for a long time).

When we caused necrosis in the epitheliums of the kidney on one side by artificial procedure it was very easy to imagine that a part of dead cells was excreted into urine and another part entered into circulation system being resorbed. *Dinkler*[37] reported examples — phthisis, peritonitis, meningitis acuta etc. — which proved influx of kidney parenchyma into the blood vessels of the kidney, and attributed it to the injury of the kidney vessels. In my experiments also, the disturbance of circulation such as hyperaemia and hemorrhage etc. in the lesion was found and kidney parenchyma which resorbed into the blood vessels acted directly at first upon the same kind of cells and influencing against the other healthy kidney led to necrosis when the irritation was too intensive. (ref. to Paragraph 1 & 2. of Chapter III.) This was to be found in the example which fell dead on the third day after the operation, and had the pathological changes produced by the injection of the emulsion of kidney cells. Moreover with the lapse of time the formation of nephrotoxin was quickened by the kidney parenchyma which entered into circulation, and both these elements, the direct action and nephrotoxin, acted upon the kidney in the other side. And when the regeneration could not happen because of too intensive degeneration, it induced proliferation of interstitial connective tissue. The changes of the kidney above mentioned told us these processes. In other words when the kidney parenchyma resorbe into the body it might give irritation and excite the funcition of the other healthy kidney to a certain degree. But if the irritation was too intensive over physiological limit, it was not hardly to suppose that degeneration would be induced. Thus I could find out that the modes of the origination were the same comparing with *H. Nakamura*'s study — the crushing of a testicle on one side induced atrophy to the other and the exstirpation of one testicle strengthened the functions of the other — and *Fujimoto*'s research — the crushing of the liver on one side produced changes in the other part.

The changes occurred on one side of kidneys when we bound ureter on the other side, were easily explained according to this reason as I have stated in the next paragraph.

2. The case of binding a ureter on one side.

The laparotomy was carried out always under the ether narcosis in order to tie up the kidney ureter on one side, and the one rabbit autopsied on the fifth day and the other on the twenty third day after the operation. The rabbit which was killed shortly after the operation decreased 250 gram in the body weight, but the other was recovered gradually with the lapse of time, and at the time of

autopsy the body weight was almost the same as before the operation.

Both examples showed albumen reaction in urine which was in the bladder at the time of autopsy.

The histologocal findings of the kidney occurred by binding ureter were already exactly examined by *Lindemann*[29] and *Ponfick*[30]; and as the object of my experiment existed rather in researching the result on the other side, I will omit to mention the findings in the operated kidney except pointing out the remarkable parenchymatous degeneration.

In the findings of the kidneys in the non-operated side the most noticeable matter was the existence of hyperaemia and hemorrhage, as the marked differences to the operated kidney and they became more conspicuous with the lapse of time. Such hypepaemia and hemorrhage were to be regarded as the changes formed by the irritation of the degenerated kidney cells on one side which were resorbed into circulations, because such remarkable hyperaemia and hemorrhage appeared also in the case of crushing a kidney on one side or of the direct action examined by injecting with the emulsion of kidney cells. The hyperaemia was in lesser degree in the example which was examined on the fifth day after the operation than in the other which elapsed more than three weeks, and this findings might depend upon the following fact that the former did not yet induce stronger degeneration or resorption of the kidney parenchyma as the latter. We could make the basis of the above statement exactly by finding the degenerating process in the kidney parenchyma on the non-operated side. It resembled so much to the findings in the directly acting case examined by the injection of the emulsion of kidney cells that there is no need to state it again, showing the image of nephrose. And moreover the example examined shortly after the operation was of slighter degree of degeneration of the kidney parenchyma than the other. *Lindemann*'s[29] and *Ponfick*'s[30] studies showed us that the functions of kidneys were continued after binding the ureter and induced atrophy gradually degenerating tubules. Because the pathological change of the kidney was of course slighter directly after the operation than that at a certain lapse of time. Hence the resorption of dead cell component was lesser and accordingly the change of other side was also slighter. In my experiment I found also that the changes of the operated kidney soon after the operation were slighter than the other.

The cause of these changes mentioned above were detailed in the preceeding Chapter. But I will discuss it in a few words. If the degeneration in the nonoperated kidney were only due to the partial phenomenon of the general intoxication which were induced by the imperfection of urine excretion, it would be impossible to explain the fact that the general symptoms of the experimental animals were almost the same as those of the healthy ones. Moreover they were different from the results which were seen in the case of insufficiency of the kidney function that is in uraemia, showing severe changes not only in kidney but also in another viscera. And on the contrary in my cases only slight changes

were to be observed in liver and hematopoietic organ etc. On the occasion of crushing a kidney, but there were no important changes to be mentioned in another viscera when a ureter on one side was bound. By the reason descrived above I can not agree to the opinion which attribute these results to those of general intoxication. Or if one supposes that the changes in another kidney were induced by the damage of nerves by the operation, I may be able to deny it according to the experiments of Dr. *Miyagawa* and others in our laboratory. Their experiments were performed on many animals under laparotomies resulting no remarkable changes in kidneys as above mentioned.

When one crushes a portion of a kidney then the part of kidney parenchyma which induces the degeneration or necrosis soon after the operation is resorbed into circulations and causes the changes directly acting upon other kidney parenchyma. I consider such a explanation is the most proper namely completely agree to Dr. *Miyagawa*'s hypothesis—"If the tissue or organ cells are parenterally injected into the organism, they act upon the homologous tissue or organ cells." In the examples which elapsed many days after the operation, the above actions became more remarkable by the increase of the degenerated kidney parenchyma in the operated kidney and then the formation of autonephrotoxin was occurred, by which the pathological changes became more intensively.

V. Experiments on nephrotoxin.

1. The case of injection of nephrotoxin under the fibrous capsule of a kidney on one side (the action of isonephrotoxin).

The immune serum was obtained from five immunized rabbits. Every time at interval of 6 to 9 days about 1.0 to 0.5 gram of kidney per kilo of body weight was made to emulsion by physiological salt solution, and injecting intraperitoneally 5 to 9 times I took the blood after a week from the last injection and separating serum I stored it in an ice box. The operation of serum injections were performed under the ether narcosis and the dose from 2.0 to 3.0 gram was injected uuder the fibrous capsule of a kidney on one side.

Stating briefly regarding the anatomy of blood vessels and lymphatic vessels in kidney, blood capillaries in the surface of the cortex communicate directly with those in kidney fibrous capsule. Moreover the lymphatic system of kidney is seen from cortex to hilus running parallel to the blood capillaries and communicate with those in fibrous capsule. So the injected serum into the fibrous capsule acted upon kidney directly by resorption from these blood and lymphatic vessels. This was the reason why I serected this place as the locality for injection to let the serum act upon the kidney on one side as rapid and intensive as possible.

I used eight rabbits for my experiments. As the symptom after the injections rabbits became low in spirits but recruited themselves on the next day, and

were killed with blows on neck or autopsied by vivisection three or nine days after injections. At this time wounds of the laparotomy were healed up and they were all lively.

The albuminuria was examined on four rabbits by urine which was in the bladder at the time of autopsy and the result was always positive.

As to the pathological findings in the injected kidneys there was no change to be found in the injecting locality except hemorrhage under the fibrous capsule only in one case. Generally the cortex had the tone of yellow colour and was turbid, but showed no hyperaemia but anaemic state. This was the constant difference between both kidneys. It was not rare to find slight depressio parenchymae in the surface of the kidney by the histological examination. This was nothing but the image which was induced by the degeneration of the kidney parenchyma and the atrophy of the tubules. The changes of the whole kidney were different in their intensities in every experiment. Some were very intensive in the cortex under the site injected with serum, some were slight in the same part, but all examples had intensive parenchymatous changes in the deeper parts. As to hyperaemia and hemorrhage it was seen, as in the macroscopic findings, no hyperaemia but rather anaemia. This was the marked difference compared with those of the direct action and also those of kidney in the opposite side. In the latter one there were hyperaemia and hemorrhage ressembling with those of the direct action. In the other words the changes of the kidney in the opposite side were different in the mode of the origination from those of the injected kidney. The parenchymatous changes of the kidney injected with serum were acute degeneration, and there is no need to mention here again about it as it was almost the same as that in the case of intraperitoneal injection of the emulsion of kidney cells. But the characteristic on this occasion was the existence of the extremly degenerated tubules gathering together besides the distribution of the degenerated ones over the whole kidney as observed in the kidney in the experiment of the direct action. And this was to be observed by the existence of depressio parenchymae or also it was evident as was showed in Fig. 3. I supposed, this might be caused by the injected serum which was resorbed rapidly by the circulation and damaged the parenchyma on this side.

In the opposite kidney some pathological changes were also perceived though they were different in their intensity. The following points are very interesting to note as the findings in both kidneys were different macroscopically, contrary to the fact that the injected kidney was turbid and rather anaemic and there induced no hyperaemia and hemorrhage showing histologically only the remarkable parenchymatous changes, the opposite kidney showed pretty well intensive hyperaemia and hemorrhage and at the same time the degeneration was perceived in the parenchym cells and these changes were almost the same as those in the experiment of the direct action (Fig. 4.). I considered that it might be regarded as the difference between the effects of nephrotoxin and the direct action of the

emulsion of kidney cells.

It might be possible to consider the changes of the kidney in the non-injected side as follows, deducing from the fact that the changes in this side were closely analogus to those in the experiment of the direct action examined by the injection of the emulsion of kidney cells. If changes such as necrosis were to be induced in kidney parenchyma in one side by the effect of nephrotoxin, a part of necrotized cell component was excreted into urine by tubules and a part of it acted directly upon the parenchyma cells on another side being resorbed into circulations. And when the direct action was slight, only increasing the physiological function, then there was no histological changes to be seen. But when the direct action was so intensive as to be over the physiological threshold, the degeneration or necrosis were to be induced to the healthy cells by the excessive irritations. This was the same as the fact that the changes were induced to a kidney on the other side by necrotizing the cells crushing a part of a kidney on one side.

In organism the cells always carry out metabolism and in this occasion a part of this cellular component is excreted out of the body harmlessly but another part of it being resorbed enters into circulations and then acts upon the homologous cells, it may cause the irritation against the healthy cells to obtain the physiologial functions and to quicken the palingenesis. Thus the various phenomena of organism are performed smoothly but if excessive necrosis of parenchym cells is to be induced pathologically at once, and the irritations which are derived from them become abnormally intensive, the homologous cells or sometimes the heterologous ones necrotized at last. And I believe my experiments mentioned above demonstrate evidently these fact.

The doubt may be properly harboured that the changes descrived above may be caused by the injection of healthy rabbit's serum. I did such a experiment with normal serum using two rabbits, but there were no changes in the kidney with naked eye. And no albuminuria appeared. Moreover the microscopical examination showed only slight hyperaemia but no parenchymatous change.

Furthermore I performed the same experiments with other organtoxin. I experimented on two rabbits with virulent isogastrotoxin (M. No. 9.) (31a). The symptom after the operation was as above mentioned. And albuminuria appeared already on the next day after the injection. Though in the injected kidney no hyperaemia and hemorrhage were to be found, it was the striking difference compared with the distinct anaemia which was induced by the injection of nephrotoxin. In the kidney parenchyma there were pycnosis of nuclei, vacuolar formation, plasmolysis, plasmoptysis and small necrotic patches in sinuous tubules and in the loops of Henle. But it was impossible to find out the necrosis of the whole epithelium of tubules as in the injection of nephrotoxin, and the degenerated cells subsisted among the necrotized ones. The kidney on the opposite side presented somewhat intensive hyperaemia and hemorrhage. The epitheliums of

the sinuous tubules and the straight ones showed distinct regressive metamorphosis in various degree and it was extremly analogous to the changes of the kidney in the opposite side in the case of the injection of nephrotoxin but the degree was slighter. Judging from these findings above mentioned I may explaine that the regressive metamorphosis of kidney parenchyma is induced by the injection of gastrotoxin under the fibrous capsule of a kidney on one side and a part of necrosed parenchyma being resorbed act upon the other side directly. And it is the very interesting question why gastrotoxin injures the kidney, and moreover this may be also the question to explain the mode the development of cytotoxin. As *Lindemann*[5] found that the intensity of autonephrotoxin was influenced by the grade of the changes of an animal's kidneys which generated it, in the generation of autonephrotoxin it seems to have a very important meaning that the kidneys induce degeneration, and nephrotoxin will be formed after the degenerated kidney parenchyma entered into circulations. And in the immunizing process to form gastrotoxin it is possible to degenerate the kidney and I have seen it as a matter of fact that the remarkable degeneration was observed in rabbits from which this gastrotoxin was obtained. Therefore nephrotoxin might exist in gastrotoxin used in this line and it induced such changes to the kidney. So I attribute the pathological changes of the kidneys which were induced by the injection of gastrotoxin to the actions of nephrotoxin which was obtained in gastrotoxin.

2. The case of injection of auto-immun (its own) serum under the fibrous capsule of a kidney on one side of experimental animals which showed the formation of isonephrotoxin (the action of autonephrotoxin).

Though my experimental animals were very few, still I have confidence that the own serum of the immunized animal was also to act upon its own kidney, when the serum was brought to the strange condition to the organ when the serum had the isonephrotoxin. The immunization was the same method above descrived. At the lapse of one week after the last injection, the blood was obtained 10.0 c.c. from the ear vein and the serum was separated immediately from it, then was injected under the fibrous capsule of the kidney on one side by the same method noted in the last chapter, then I killed them with blows on neck and autopsied on the fifth and on the seventh day after the operation.

The symptoms after the operation had of no note worthy, and were as vigorous as healthy ones.

At autopsies there were no changes in the injected places. Comparing with both kidneys in the section there were some different findings. The injected side showed obvious anaemia and was turbid but in the opposite side slight hyperaemia and hemorrhage were to be found. But they were very slight comparing with those which were to be found in the opposite side in the case of injection of isonephrotoxin into a healthy rabbit. This might be caused by the

existence of the degeneration in the kidney parenchyma in marked degree by both the direct action of the emulsion of kidney cells and isonephrotoxin before the serum injection but I suppose there might be generated something as anticytotoxin.

As to the histological findings there was necrosis of parenchym cells in the injected side and nucleary casts here and there in the straight tubules which were generated by the desquamation of cells. In the sinuous tubules intensively degenerated epitheliums were to be observed, and in the lumina albumen masses and desquamated cells were to be found. These degenerated tubules assembled together. This change was also to be observed in the injected side of isonephrotoxin.

In the opposite kidney hyperaemia was more remarkable comparing with that of injected side and also hemorrhage was to be observed, with other various changes as there in the case of repeated injections of kidney cells emulsion (ref. to Paragraph 3. of Chapter III.). In these examples localized proliferation of the connective tissue and the round cell infiltration were to be noted as already mentioned in Paragraph 3 of Chapter III. In short the difference of the changes in the kidneys on both sides as above noted showed as follows: the injected side was influenced by mass action of nephrotoxin which was brought in mass under its fibrous capsule and consequently acute degeneration was occurred. The non-injected side was acted directly by the resorbed dead cell component of the injected one. It is clear that the immunizing process with the emulsion of homologous kidney cells created not only isonephrotoxin but also at the same time autonephrotoxin.

VI. Summary.

I can summarize as follows from the experimental results above mentioned.

The intraperitoneal injection of the emulsion of homologous or heterologous kidney cells into rabbits showed various general symptoms. (I injected the emulsion of kidney cells of rabbit, ox and horse into rabbits.) They differed in intensity according to the quantity of injected emulsion of kidney cells or by its variety.[27] In my experiments the reaction of the emulsion of heterologous kidney cells was more slight and the lethal dose was much than that of the emulsion of homologous kidney cells. Of course on according to the intensities the pathological findings were also various. The most remarkale histological changes were to be observed in the kidneys and there was the parenchymatous degeneration in every where. Moreover we always noted the appearance of albuminuria. There were always some changes in other viscera — that is liver, suprarenal glands etc., but not important to be mentioned in comparing with those of kidneys. The changes in the lungs, the digestive duct and the hematopoietic organs were indefinite and slight. As above noted, the parenteral injection of the kidney tissue

into rabbits undoubtedly acted upon the kidney tissue. Bringing about the artificial injuries by crushing a part of a kidney on one side or by binding a ureter on one side, the severe pathological changes presented in the operated kidney parenchyma. It is an interesting and important problem to examine by such procedures the inducement of the pathological changes on the other non-operated kidney. And here intensive pathological changes were also to be seen and they were entirely the same in their essence as those produced by parenteral injection (intraperitoneal injection) of the emulsion of kidney cells. I may concluded as follows from these two experimental results above mentioned.

If there is the degeneration or the necrosis in the kidney epitheliums by various causes, a part of the cells will be excreted into urine but the other part will enter into circulations being resorbed and act upon kidneys at the first time. If a great quantity of cell component is resorbed and accordingly its action is too intensive, then the effect will induce degeneration and necrosis in the epitheliums of the other healthy kidney. If the irritative effect is not so intensive, there are not only abnormal changes as degeneration, but will quicken the function of the epithelium. These phenomena are carried on always in organism, and the substitution of the kidney epithelium, the resorption of this cell component and the irritative effect on the other healthy parts are to be regarded as the real physiological stimulant which hold the excretion of urine in normal condition. I never intend to assert that the excretion of urine is carried on only by the effect of the resorbed cell component. But I consider that the direct action of the resorbed cell component have an important rôle in the physiological process. In my experiments I intended to examine the direct action by letting the physiological irritation to act more intensively over the physiological threshold.

The parenteral injections of kidney cells emulsion (iso- or heterologous) into rabbits form nephrotoxin after the definite period of incubation (about more than one week). I have never heard the experiment to examine cytolysis by the injection of nephrotoxin under the fibrous capsule of a kidney on one side, and by this experiment I found the intensive parenchymatous degeneration and necrosis in the injected kidney. Such effects were to be found equally in immunized animals themselves (the effects of autotoxin), but in this case the effects of nephrotoxin were more slight than in normal rabbits. An antinephrotoxin would be formed at the same time of the procedures of immunization, and the effects of autoserum would be weakened by this anticytotoxin. But it is very difficult to suppose the formation of such an anticytotoxin. When the emulsion of kidney cells is injected repeatedly into an animal at regular interval, the clinical symptoms is to be weakened gradually with the lapse of time. I suppose that the rabbit was not only accustomed to the emulsion of kidney cells but at the same time there might form an antibody which would protect gradually such an irritation.

When the necrosis of parenchyma in a kidney on one side is induced by the action of nephrotoxin, it is very interesting to examine the changes of the

kidney on the other side. Namely we found in the opposite side somewhat intensive degeneration and necrosis. And these findings were entirely the same as those of the rabbit which was injected the emulsion of kidney cells. So I concider that the kidney epitheliums on one side induced the degeneration and necrosis by the effect of nephrotoxin and the components of cells being resorbed brought the direct action upon the other side and the changes of the kidney by the effect of nephrotoxin and those of the opposit one were different macroscopically and histologically. In the former I could find out conspicuous anaemia but in the opposit side there conspicuous hyperaemia and hemorrhage were to be found. But regarding the changes of parenchyma it was very hard to distinguish both as I found only degeneration and necrosis.

Now the repeated intraperitoneal injections for a long time which were performed once a week with a definite quantity of rabbits' kidney cells emulsion to healthy rabbits produced always after six, or ten weeks the conspicuous proliferation of the interstitial connective tissue of kidney and I could percieve in the typical case the image of the secondary granular kidney and intensive atrophy of the parenchyma. I concider that these atrophy of parenchyma and proliferation of interstitial connective tissue are induced by both actions — that is the direct action and the action of autonephrotoxin — which are more strongly over the process of regeneration.

I have next to explain whether the phenomena of these direct and immune body actions above mentioned exist in reality during a certain diseases or not.

An example is the nephritis in diphtheria. I suppose that the kidney epitheliums are subjected to the degeneration and necrosis by the toxin which is ejected from diphtheria bacillus. At the time of resorption of these degenerated cell component, it is not so hard to imagine that there occur the direct action and then the effect of nephrotoxin. Thus the image of nephritis becomes to be observed equally on both sides. And if there appeares necrosis of cells, for instance as in tuberculose nephritis on one side, the irritative action will influence to the kidney on the other side and the compensatory functions will act remarkably. But when the desolation becomes too intensive it will induce parenchymal changes on the other side. The nephrectomy performed on such an occasion, stimulates at first intensively the compensatory functions.

From the above mentioned results I can decide the important rôle of the direct action of the kidney cells which are resorbed parenterally and the effect of nephrotoxin for the promoting of nephritis.

VII. Conclusion.

1. The intraperitoneal injection of the kidney cells emulsion of rabbit, ox and horse into rabbit always induce the degeneration or necrosis in the kidney parenchyma. Various changes are to be observed in other viscera but not so intensive as in the kidneys.

2. The repeated parenteral injections of the emulsion of homologous kidney cells into rabbit for a long time, atrophy eventually the parenchyma and make the secondary chronic interstitial nephritis causing the proliferation of the interstitial connective tissue.

3. When we crush a part of a kidney or bind the ureter on one side it induces the degeneration in a kidney on the other side and the necrosis when the reaction is intensive. From these experimental results, the resorbed component of degenerated kidney parenchyma causes the irritative effect upon the healthy part and at last produce degeneration, when the irritative effect is over the physiological limit, as if in the case of parenteral injection of the kidney cells emulsion.

4. I considered that the above described action is produced by the resorbed cell component and regard it as the direct action as Dr. *Miyagawa* stated and I am agreed to distinguish it from the action of nephrotoxin.

5. As many literatures teach us, in the bodies of rabbits which are immunized with the kidney parenchyma, nephrotoxin is formed after about one week as the period of incubation.

6. The remarkable pathological changes are to be observed in the kidney of healthy rabbit after the injection of isonephrotoxin under the fibrous capsule. And the changes which injure the kidney are most intensive among those of the other viscera, showing the special affinity of nephrotoxin to the kidneys.

7. When we made the injection of nephrotoxin into the immunized rabbit under the fibrous capsule of a kidney, which showed the formation of isonephrotoxin, we found there the remarkable cytolysis in the injected kidney, that is the effect of the mass action of autonephrotoxin.

8. When the necrosis of kidney cells is induced by injection of nephrotoxin under the fibrous capsule on one side, and when a large quantity of kidney parenchyma is resorbed into circulations, it is possible to generate the direct action on the other side. From histological point of view it is difficult to distinguish the effect of nephrotoxin from that of the direct action, the injected kidney with nephrotoxin shows, however, no hyperaemia and hemorrhage but rather anaemia. On the contrary, the kidney on the other side acted by the direct action shows marked hyperaemia and hemorrhage. As to the parenchymatous pathological changes, both kidneys show regressive metamorphosis.

9. The direct action of the resorbed cell component and the formation of nephrotoxin are always going on during whole life of organism and I consider that the moderate irritations are necessary to maintain the physiological function of kidney.

10. The direct action of resorbed kidney parenchyma and the effect of nephrotoxin are to be found when the necrosis of the kidney epitheliums is induced in progress of verious diseases, and I consider that the interstitial inflamation will consequently follow when the changes are too intensive. So I believe firmly that

it is necessary to take into consideration the direct action and the effect of the nephrotoxin in order to certify the image of nephritis occurred by various causes.

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Explanation on figures.

Fig. 1. A part of the kidney which was injected with the emulsion of ox kidney cells. The epitheliums swell up, the protoplasm differs in the degree of staining here and there and vacuoles are dotted. The nuclei show pycnosis and karyolysis. In the lumina there exist albumen lump and stripped degenerated nuclei. (Stained with hematoxylin and eosin, obj. Immersion 1/12, oc. 4.)

Fig. 2. Section of the medulla of a rabbit's kidney which was injected with the emulsion of horse kidney cells. Several tubules in Henle's loop are degenerated, and seen filled with nuclear casts. (The same staining as Fig. 1. obj. Zeiss D. D. oc. 4.)

Fig. 3. Section of the medulla of the kidney injected with nephrotoxin. Almost the same changes pictured in Fig. 2. are to be observed except hyperaemia and hemorrhage. (The same staining, obj. Zeiss D. D. oc. 4.)

Fig. 4. Section of the kidney on the opposite side of Fig. 3. Hyperaemia is more remarkable comparing with Fig. 3. Besides, in the sinuous and straight tubules intensive degeneration and necrosis are seen. (The same staining, obj. Zeiss A.A. oc. 4.)

Fig. 1.

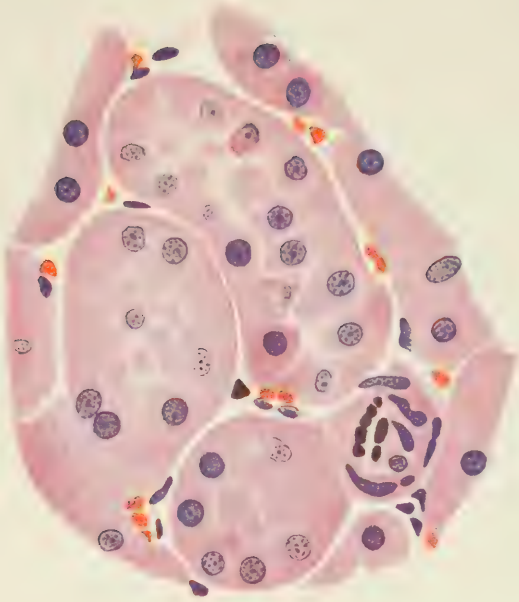
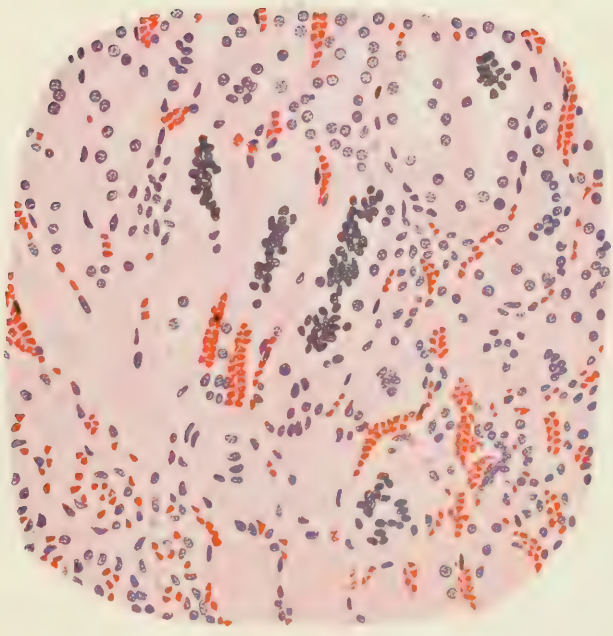


Fig. 2.



(Dr. J. Kimura : A Study on Kidney cells as toxin or irritative Substance etc.)

Fig. 3.

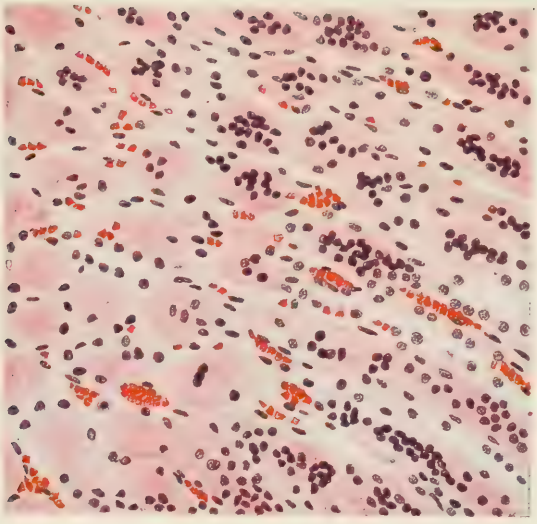
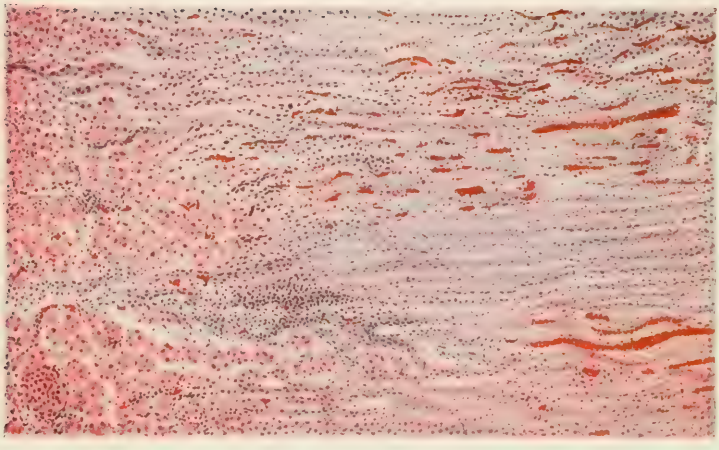


Fig. 4.



(Dr. J. Kimura : A Study on Kidney cells as toxin or irritative Substance etc.)

Experimentelle Untersuchungen über die Lymphocyten.

Von

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(Veröffentlichted in Japanischen in the Japanese Journal of Experimental Medicine Vol. V. No. 3, 1921.)

Der Verf. injicierte den gesunden Kaninchen subcutan und intraperitoneal eine Emulsion und ein Autolysat der Lymphdrüsen des Kninchens und des Rindes und studierte den Einfluss auf das Blut und das haematopoetische Organ. Die Resultate lauten wie folgende:

1) Bei der Injection von grosser Menge derselben trat als eine specifische Reaction nach 24 Stunden eine Abnahme der weissen Blutkörperchen, besonders der Lymphocyten auf, während die Zahl der Roten, der basophilen, der eosinophilen Leucocyten und der grosse Mononucläeren eine unbedeutende Schwankung zeigten. Bei den anatomischen und histologischen Untersuchungen fand der Verf. dabei immer verschiedene regressive Veränderungen im ganzen lymphatischen System, nämlich Nekrose, Pyknose, Zerfall und Schwund der Lymphocyten sehr auffallend waren, wobei eine ausgesprochene Fettablagerung in anderen Organen, d. h. in der Leber, der Niere, der Milz, den Hoden, der Lunge, der Aorta und dem Skelletmuskel in Betracht kam. Dazu wurde Erscheinung der Fettwanderung im Körper wahrgenommen und ihre Stärke hielt immer mit dem Untergang des lymphatischen Systems, bezw. der Lymphopenie gleichen Schritt. Also möchte der Verf. eine innige Beziehung der Lymphocyten zu dem Fettwechsel annehmen.

2) Die oben erwähnten hämatologischen u. histologischen Veränderungen waren mehr auffallend bei der Autolysatinjektion als bei der Injektion der Lymphdrüsenemulsion, respectiv heterologene als homologe, intraperitoneale Injektion als subkutane, tägliche Darreichung als bei den mit Pausen.

Wobei spielen die Art und die individuelle Disposition der Tiere eine Rolle auf das Auftreten dieser Veränderungen.

3) Bei den Fällen, wo die Injection in geringer Menge derselben ausgeführt wurde, kam eine Leucocytose mit pseudocosinophilen Leucocyten und manchmal eine Lymphocytose zum Vorschein; anatomisch wurden dabei eine Anschwellung des Lymphatischen Systems, histologisch sowohl geringe regressive als such regenerative Vorgänge betrachtet.

4) Bei den Kontrollversuchen, wo die Injection in geringer Menge der Knochenmark- und der Milzemulsion ausgeführt wurde, fand der Verf. eine deutliche Zunahme der Weisen, besonders eine Lymphocytose und eine ausgesprochene Anaemie, dabei war anatomisch und histologisch kein Zeichen der Atrophie im lymphatischen System, sondern eine Hyperplasie zu sehen, aber bei der Injection von der Leber- und der Nierenemulsion solche Veränderungen sehr undeutlich.

Bei den Injectionen der Knochenmarkemulsion, und zwar in grosser Menge fand der Verf. eine regressive sowie eine regenerative Veränderung in der Myelopoëse und eine Degeneration in der Erythropoëse.

Auf Grund dieser Versuche behauptet der Verf. dass bei der Injection der Emulsion verschiedener Organen eine Organemulsion speciffisch auf dasselbe Organ wirkte und die oben erwähnten Befunde nicht als eine einfache cytotoxische Erscheinung zu betrachtet sei, wie man sie bei der Injection von Lymphotoxin, Myelotoxin und Splenotoxin sieht.

(*K. Seto.*)

A Clinical and Experimental Study of Lymphocytes.

By

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clinical Director: Dr. Yoneji Miyagawa.
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Contents.

I. Introduction.
II. Description of the large lymphocyte with abundant protoplasm.
III. Clinical obseration.
  1. Lymphocytes in healthy individual.
  2. Lymphocytes in various non-febrile chronic diseases.
  3. Lymphocytes in tuberculosis.
  4. Lymphocytes in typhoid fever.
  5. Lymphocytes in other various acute infectious diseases.
  6. Summary of clinical observations.
IV. Experimental study.
  1. Lymphocytes in the normal rabbit.
  2. Influence of typhoid vaccine on Lymphocytes.
  3. General view of the appearance of large lymphocytes with abundant protoplasm.
  4. Influence of the preparation of lymphatic gland on blood lymphocytes.
  5. Influence of the autolysate of bone marrow and spleen cells on blood lymphocytes.
V. Summary.
VI. Conclusion.

I. Introduction.

The majority of lymphocytes in the normal blood are the smallest of the white cells and as a rule in size as small as red blood cell. They are generally called the small form. But somewhat large lymphocyte also appears in the blood in few numbers. Both of these small and large forms have a very large nucleus in relation to the size of the protoplasm. The nucleus is usually located in the

centre but may have an eccentric position. It is compact or closely reticulated and is strongly stained. The protoplasm of these cells is usually in the form of a narrow rim surrounding the nucleus and showing a strongly basophilic homogeneous cytoplasm.

Besides these usual lymphocytes we occasionally find characteristic lymphocytes, their protoplasm being very large in extent and showing slightly basophilic staining properties. I have thrown my attention to the fact, that the large lymphocytes characterized by abundant protoplasm are demonstrable in extraordinary great number under certain pathological conditions, although we have found a few of them up to present in the normal blood.

The abnormal abundance of protoprasm of blood cells is generally considered as a little clinical significance, except as a probable sign of age of the cells. *Pappenheim*[1] and many other authers regarded the large lymphocyte with abundant protoprasm simply as an old example, while *Naegeli*[2] stated that the age of the cells rather depends upon the peculiarity of nucleus than that of protoprasm under some pathological conditions *Schleip*,[3] *Ziegler*,[4] *Schling*,[5] *Naegeli*[2] and others took notice of such large lymphocytes in the blood stream whithout any futher study.

Recently *Bergel*[6] has reported that when tubercle bacilli or lipoid substances were inoculated into the abdominal cavity of a mouse, mononuclear cells with abundant protoplasm may be demonstrable numerously in the peritoneal exsudates of this animal after the lapse of some times. He recognized these cells as "working form" of lymphocytes which make ameboid movements and possess phagocytic power, and distinguished them from the usual small lymphocytes with a narrow rim of protoplasm surrounding the nucleus, which he called "resting form." He considered also that the both forms of these lymphocytes may be changed into shape of each other according to circumstances.

More recently, however, *Aschoff* and *Kamiya*[7] have expressed that the most of *Bergel*'s so-called "working form" of lymphocytes belong not to the true lymphocytes but to histiocytes.

Lymphocytes, in general, may easily crushed. Fomerly the so-called basket cells or "shadows" (first described by *Gumprecht*) were considered as the degenerated forms of lymphocytes. The "shadows" arise, however, by faulty technik, especially by the pressure in making blood smears. Some of them are indicative of lowered vitality but the majority are recognized as fragmentations which are due to feeble power of resistance of young cells, because reliable nucleoli are detected very frequently in them (*Askanaszy*,[8] *Fraenkel*,[9] *Grawitz*,[10] *Naegeli*,[2] *Morawitz*,[11] and others). Although antecedent investigators have deserived that the "shadows" are demonstrable numerously under some pathological conditions, according to my examination, contrary to their findings, there is no definite difference in the number of the "shadows" between pathological and physiological blood. But I noticed that numerous shadows are always found in

children suffering from typhoid fever or whooping cough. Besides such changes of lymphocytes, I have noticed that the pycnosis seems to have more clinical significance than the shadow. The pycnotic lymphocytes appear as deeply stained, structurless, compact mass with crenated edge. It shows a great variety of forms, being round, oval, or irregular shaped bodies. They are easily seen associated with diminution in the number of lymphocytes during acute stage of infectious diasease. I have examined carefully in many cases but I could not established neither parallelism nor contradiction between the so-called shadows and pycnotic lymphocytes. It seems probable that no relationship exists between them.

This present investigation was under-taken for the purpose to determine under what condition the large lymphocytes with abundant protoplasm as well as pycnotic lymphocytes appear in the blood and whether any definite relationship exists between them.

II. Description of the large lymphocyte with abundant protoplasm.

This form of lymphocytes is distinguished from the usual small lymphocyte by nucleus-protoplasm-relation i. e. the protoplasm of this cell is abnormally abundant in proportion to the size of the nucleus. The cells therefore about three or four times of a usual small lymphocyte in size. The nuclei, in which a distinct nucleolus may be often seen, are generally oval, sometimes round or are seen as the so-called kidney type. The nuclei are always sharply outlined and usually eccentric in position. They are compact or closely reticulated and relatively poor in chromatin, but are strongly basophilic, although less so than are the nuclei of the small lymphocytes. The protoplasm of these cells makes a broad zone, surrounding the nucleus, and showing usually homogeneous feeble basophilic qualities. They have regular or sometimes irregular fragmentary outline. The protoplasm of older cells show often some difficulty in staining, and occasionally exhibit slightly stained coarse net-like structure. The so-called azur-granules are sometimes seen in this protoplasm by the Giemsa stain. The oxydase-reaction is absolutely negative.

The large lymphocytes with relatively great protoplasm resemble to the large mononuclear leucocytes in the stained form. It is however not difficult to make a distinction between these two cells by the excellent giemsa stain. In smear, stained by giemsa, the nuclei of the large mononuclear cells are not always so sharply outlined. They are relatively poor in chromatin. The protoplasm shows very fine reticulum with nodalthickings which are somewhat strongly and may give an appearance of granulations, while the protoplasm of large lymphocytes show not only pale and homogeneous appearance even when they are strongly stained, but also there is a perinuclear clear zone which, as well known, seems to be characteristic of the lymphocytes. In other words these lyphocytes are differentiated from the large mononuclear cells by showing a distinct clear pale

rim surrounding the nucleus, which is thrown into relief by the deep staining of the nuclear substances.

From the morphological point of view, the large lymphocytes with abundant protoplasm are similar to Kiyono's histiocytes[12] as well as to the large mononuclear cell. But no auther has yet proved that the histiocytes appear so numerously in the peripheral blood circulation. In order to determine if the large lymphocytes noticed by me would be histicytes or not, I have inoculated typhoid vaccine into the rabbit which was treated proviously by repeated administrations of carmine. Then after certain periode, the large lymphocytes appeared in great number in the blood obtained from the ear vein, as it is stated in the chapter of experimental study, and no carmine cell — histiocyte — was found in the peripherical blood but found only in the venous blood of liver and spleen.

In addition to these examinations I have proved in my large lymphocytes the Altmann-Schridde's fuchsinophilic granules, which are always demonstrated in the characteristic locality and arrangement in the protoplasm.

From these examinations it is evident that the large lymphocytes which called my attention belong to the true lymphocytes, being neither the large mononuclear leucocytes nor the histiocytes.

III. Clinical observation.

Clinical observations were undertaken to know the exact conditions which favour the appearance of the large lymphocytes with abundant protoplasm and the pycnotic lymphocytes in the blood. For this purpose it is necessary to count differently three types of lymphocytes — usual or normal lymphocyte, large lymphocyte with abundant protoplasm and pycnotic lymphocyte — and to make a study of comparing their numbers in various cases under normal and pathological conditions.

The examinations were performed always before meals, and blood obtained from the ear for the purposes of making films and doing counts. Blood films were stained by giemsa, and for counting of leucocytes Thoma-Zeiss counting chamber was employed.

The distribution of leucocytes in the films is not always uniform, the polynuclear cells and the large lymphocytes being relatively numerous in the edge, and the small and the pycnotic lymphocytes, in the middle zone of the films. And I have endevoured to obtain comparatively accurate results.

1. Lymphocytes in healthy individual.

The investigation was carried out in 12 cases of adult, 6 males and 6 females. No remarkable difference in the number of lymphocytes was observed between the both sexes. The large lymphocytes with abundant protoplasm were found to be less than 300 per cubic millimeter in 7 out of 12 cases. The average numbers

of total leucocytes and of the various types of lymphocytes are shown in the following table.

	Total leucocytes	Total lymphocytes	Total lymphocyte	Large lymphocyte	Pycnotic lymphocyte
Number per cmm.	7700	2300	2100	190	10
Percentage		29.8	27.2	2.5	0.1

2. Lymphocytes in various non-febrile chronic diseases.

Differential counting of lymphocytes were performed in 14 cases presenting neither subjective nor objective severe symptom i.e. secondery syphilis, stomach cancer, hysteria, diabetes mellitus, paroxysmal haemoglobinuria, habitual constipation, bronchial asthoma, anaemia, heart-disease, tabes dorsalis, epilepsy, and morbus basedowi.

According to the kind of disease, to the stage and to the case of the same kind of disease, there were found some difference in the number and percentage of lymphocytes. But I have noticed in almost all cases, as a rule, a slight increase in the number of lymphocytes as in healthy. And also the large lymphocytes with abundant protoplasm were found numerously in all except 3 cases. Therefore, total numbers of lymphocytes was, in general, higher in chronic diseases than in normal individuals. The pycnotic lymphocytes were observed in 5 out of 14 cases.

The following table shows the average number in comparison with normal blood.

	Total leucocytes	Total lymphocytes	Normal lymphocyte	Large lymphocyte	Picnotic lymphocyte
Chronic disease	7300	2700	2030	630	40
Normal blood	7700	2300	2100	190	10

3. Lymphocytes in tuberculosis.

The examinations were performed in following 33 cases; pulmonary tuberculosis, tubercular pleurisy and peritonitis and tubercular meningitis.

Clinical symptoms of pulmonary tuberculosis are not all alike. I have attempted, therefore, to divide pulmonary tuberculosis into following four groups: 1. Early stage; a few physical signs, patient complains of nothing except slight fever. 2. Secondary stage (described by *Turban*) with tendency to recover; at times a few coughs or mild symptoms. 3. Secondary stage with progressive tendency. 4. End stage or severe case.

The results of the differential count of lymphocytes are presented in the

following table in which a comparison has been drawn up between the various tubercular conditions of disease.

		Total leucocytes	Total lymphocytes	Normal lymphocyte	Large lymphocyte	Pycnotic lymphocyte
First group of pulmonary tuberculosis (Average number of 6 cases)		7730	3170	1980	1150	40
Second group of pulmonary tuberculosis (Average number of 6 cases)		8480	3720	2280	1420	20
Third group of pulmonary tuberculosis (Average number of 3 cases)		8200	1900	1240	380	280
Fourth group of pulmonary tuberculosis (Average number of 9 cases)		12955	1230	770	200	260
Pleurisy & peritonitis (in convalescence) (Average number of 4 cases)		8900	3230	2080	1060	90
Tubercular meningitis (Average number of 5 cases)		18600	1730	1070	80	580
Contrast	Chronic diseases (Average number of 14 cases)	7300	2700	2030	630	40
	Normal individual (Average number of 12 cases)	7700	2300	2100	190	10

As *Rubino,*[13] *Steffen*[14] and others stated, I have recognized also the increase in the number of lymphocyte in the early stage and in the mild cases, while on the contrary in the end stage and in the severe cases, lymphopenia. The following conclusion is drawn from the summary of results obtained: Lymphocytosis is due to the increase in the number of large lymphocyte with abundant protoplasm and lymphopenia is always associated with the appearance of pycnotic lymphocyte.

4. Lymphocytes in typhoid fever.

Generally speaking the typhoid fever run the characteristic course devided into stadium incrementi, stadium aknes, stadium decrementi and convalescence. Since Naegeli's exact systematic studies it is a well known fact that definite haematological changes are seen during its course. In the present study I could not always examine continually the blood pictures from oncet to recovery of the same case, so that I had to arrange numerous different cases examined, in four groups of the four stages. I will compare them with each other and illustrate what changes of lymphocytes occur in the course of this infectious disease.

(1) Lymphocyte in stadium incrementi. The total number was reduced to the minimum on the fourth or the fifth day after the onset of disease, averaging about 900 per cubic millimeter, and their average number of 18 cases in this stadium was only about one half of that of healthy individuals. The number of large lymphocyte with abundant protoplasm was diminished from the third to the fifth day, the average being about 30 per cubic milimeter, while they suddenly increased in number from the sixth or the seventh day, amounting to 390 in average, that is about twice the normal standard. In this stage the pycnotic lymphocytes became numerous and were seen in every case, the average number being about 200 per cubic millimeter.

(2) and (3) Lymphocyte in stadium aknes and decrementi. The total number of lymphocytes began to return to norm step by step, together with a remarkable increase in the number of the large lymphocytes. In severe cases they were reduced again at the end of stadium aknes. The reduction was very distinct, being as low as about 700 or less per cubic millimeter, that is one third of the number under normal condition. The pycnotic lymphocytes were seen usually in small number, but they increased in severe cases.

(4) Lymphocyte in convalescence. As defervescence went on, lymphocytes became very mumerous, their absolute number being in average from 3000 to 4000 per cubic millimeter, about one fourth of which were occupied by the large lymphocytes. The average number of the latter in 35 cases amounted to 785 per cubic milimeter, that is about four times as much as the normalstandard. Associated with this lymphocytosis, the pycnotic lymphocytes decreased markedly in number, but they were found in 20 out of 35 cases.

The findings are summarized in the following table.

	Total leucocytes	Total lymphocytes	Normal lymphocyte	Large lymphocyte	Picnotic lymphocyte
In the first stage (Average number of 18 cases)	5220	1110	783	122	205
In the second & the third stage (Average number of 39 cases)	4740	1300	667	450	183
In convalescence (Average number of 35 cases)	7000	3300	2405	785	110

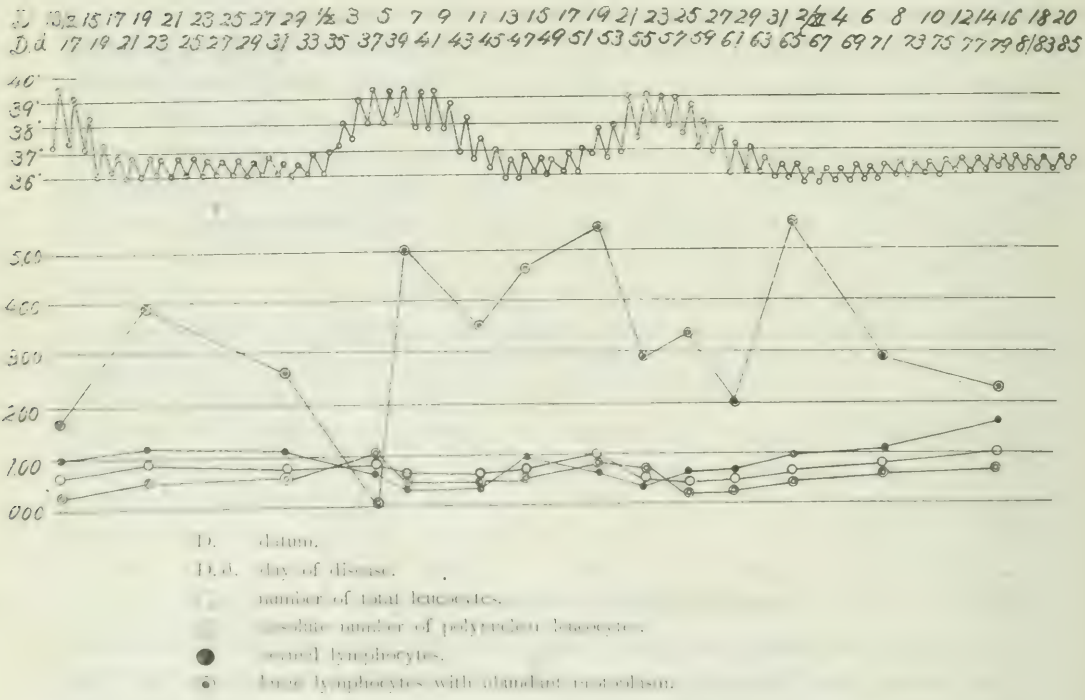
As this table shows, in typhoid fever I found quite characteristic changes of the various types of lymphocytes. During its first stage the large lymphocytes did not only increase but diminished in number, while the picnotic lymphocytes showed progressive rise in number. Contrary to this, in convalescence the former became very numerous, while the latter were reduced.

Such typical change of lymphocytic figure were observed distinctly in a case which relapsed three times. In the convalecence of the first attack in this case the large lymphocytes with abundant protoplasm became numerous and amounted

to 12 per cent. of the total leucocytes. They disappeared, however, very rapidly at the beginning of the secondary attack, while the pycnotic lymphocytes increased in number, reaching as high as 1.5 per cent. of the total leucocytes. At the beginning of the third recurrence I found again similar in lymphocytic picture, the large lymphocytes diminishing from 900 to 550 per cubic millimeter and the pycnotic cells increasing from non to 440 per cubic millimeter. In the last convalescence, however, the large lymphocytes increased again in number, while the pycnotic cells diminished.

Judging from these results, the number of large lymphocytes are very labile. It is shown in the curve I, how labile they are. In order to illustrate evidently, the change of leucocytic picture of the last case, the curve is drawn according to Miyagawa's idea. It indicates the proportion of the absolute number of the total leucocytes and each different leucocyte in the course of disease to their normal standard (cf. p. 369).

Curve I.



D. datum.
D.d. day of disease.
number of total leucocytes.
ordinate number of polynuclear leucocytes.
normal lymphocytes.
large lymphocytes with abundant protoplasm.

5. Lymphocytes in various acute infectious deseases.

Lymphocytes were examined in 22 cases of various acute infectious disease, including one or several cases of influenza, whooping cough, scarlet fever, dysentery, epidemic meningitis, lethalgical encephalitis, puerperal fever, appendicitis and catarrhal jaundice. The results obtained from these various acute diseases show

that, although some differences were observed in each case, lymphocyte changes were similar in principal feature to those in cases of typhoid fever. That is, in the early stage or in the severe case the pycnotic lymphocytes were seen in considerable number and in the later stage an increase in the number of the large lymphocytes was observed. The increase of the latter took place in parallel to so-called post-infectious lymphocytosis.

6. Summary of clinical observations.

(1) A definite change of lymphocytic picture may be seen both in the early and in the late stage of acute infectious diseases.

(2) The large lymphocytes with abundant protolasm which appear in a few number in normal blood become numerous in mild chronic diseases and in the convalescence of acute febrile diseases.

(3) On the contrary, the pycnotic lymphocytes are seen in acute severe stage associated with lymphopenia.

The summary is well shown in the following table.

		Total lymphocytes	Large lymphocyte	Pycnotic lymphocyte
Normal standard (Healthy individual)		2300	200	10
Lymphocytosis	in the convalescence of typhoid fever	3300	800	130
	in the mild or in the early stage of tuberculosis	3200	1200	40
	in the second group of pulmonary tuberclosis	3700	1400	20
Lymphopenia	in the tubercular meningitis	1700	100	580
	in the severe pulmonary tuberculosis	1200	200	260
	in the early stage of typhoid fever	1100	100	160
	in the 2nd and 3rd stage of typhoid fever	1300	450	180

IV. Experimental study.

The present investigation was undertaken in order to determine experimentally, whether the large lymphocytes with abundant protoplasm may increase in number or not under certain treatment. I have used rabbit as experimental animal. It is necessary to know previously the normal standard number of blood cells in normal rabbit.

1. Lymphocytes in the normal rabbit.

The lymphocytic picture was examined in all the rabbits used, before the experiment. The average number of total leucocytes and various lymphocytes in 29 normal rabbits are given in the following table.

	Total leucocytes	Normal lymphocyte	Large lymphocyte
Number per cmm.	10400	4500	240
Percentage		43.1	2.3

As the table shows, there is no great difference between normal rabbit's blood and human blood in the number of large lymphocytes with abundant protoplasm.

2. Influence of typhoid vaccine on lymphocyte.

It is recognized by many investigators that the animal administered with typhoid vaccine shows blood picture similar to that in a patient suffering from typhoid fever. I have specially examined lymphocytic changes.

The procedure. The experiment was carried out in 5 cases by following method;

1. Typhoid vaccine was received from our own institute.
2. Dose administered was varied in each case (0.02, 0.05, 0.07, 0.1 or 0.15 cc. per kilogram of body weight.)
3. Adninistration was performed intravenously in the left ear.
4. Blood sample was obtained from the vein of the right ear for purposes of making films and doing counts.
5. Blood films were stained with giemsa's solution.
6. Blood examination was repeated in each case over and over again in the 5th, the 10th, the 15th, 30th minute, the 1st, the 2nd, the 3rd, the 4th, the 5th, the 6th hour, the 2nd, the 3rd, and the 4th day after the inoculation.

Results obtained were various according to the dose of administration of vaccine.

1. Total number of white blood cells was reduced immediately after the inoculation and this leucopenia continued for one to four hours, after which leucocytosis was produced. The degree of the decrease or the increase in the number of leucocytes depended, as a rule, on the dose of administration of vaccine and the change in the number of leucocytes, was largely referable to the polynuclear cells.

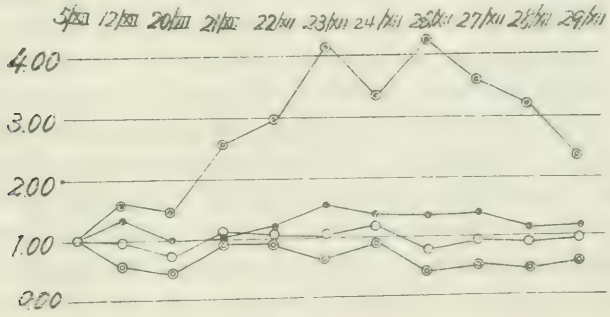
2. Number of lymphocyte. The lymphocyte was usually normal or slightly reduced in number after the inoculation of 0.05 or 0.02 cc. of vaccine per kilogram of body weight, although the leucopenia became remarkable. When

0.07 cc. or more of vaccine per kilogram was inoculated then lymphopenia was produced in moderate degree. About five hours after the inoculation when the leucocytosis had reached its highest limit, the lymphocyte began to resume slowly its normal number. After the laps of one or two days, total number of leucocytes returned to the normal standard while lymphocytes showed a progressive rise in number untill the lymphocytosis reached the highest limit from the third to the fifth day.

3. Large lymphocytes with abundant protoplasm. Associated with lymphopenia they disappeared after the inoculation for some time. But one or two days afterwards, when lymphocytosis occured, they rose to above normal number. It is a very interesting fact that the large lymphocytes became numerous only when lymphopenia was noted in the early course. When the inoculation was repeated at the time when the large lymphocytes became numerous, they disappeared again promptly, but they increased the next day in large number than before. The same change of lymphocytic picture as this I found clinically in a patient suffering from typhoid fever, in which the relapse was repeated three times (this fact was stated in the chapter of clinical observation.). Also in a case of the present experiments I could succeed to make the large lymphocytes so numerously by four times repeated inoculations, that their number amounted to 1334 per cubic millimeter. This number of the large lymphocyte was about fifteen and a half times greater than that under normal condition of the animal. When the injection of 0.07 or 0.1 cc. of vaccine per kilogram of body weight was repeated four times at the interval of a day into a rabbit previously immunized against typhoid vaccine, the animal showed blood picture similar to that of a patient suffering from typhoid fever. Although the injection was not repeated any more, the large lymphocytes appeared in a large number for some time, as if in the convalescence of typhoid fever.

The following curve shows leucocytic picture of the latter case. The principle of drawing of the curve is the same as that of curve I.

Curve II.



It is concluded from these results that no increase of the large lymphocytes in number can occur without the foregoing stage of lymphopenia. The increase of these cells in number becomes considerable when the injection of vaccine is daily repeated in a definite dosage enough to produce lymphopenia for some period after the injection.

3. General view of the appearance of large lymphocytes with abundant protoplasm.

The physiological functions of organs and tissues is going on when a definite equilibrium exists between formation (progressive change) and destruction (regressive change), as well as between stuff-exchange (assimilation and dissimilation) of cells which constitute the organs or the tissues. Disturbance of these equilibriums may cause biological reactions to run unexpected course.

According to my own clinical observation and experimental study, during the acute stage of a disease, lymphocytes are reduced in number and at the same time pycnotic lymphocytes become demonstrable. Contrary to this, lymphocytosis associated with the appearance of the large lymphocytes becomes remarkable in mild chronic diseases and in convalescence of acute febrile diseases. Similar changes of lymphocytic picture are experimentally observed. And I have come to the conclusion that no increase of the large lymphocytes in number occurs without the foregoing stage of lymphopenia.

From this fact, it seems probable that although the lessened reactivity of the lymphocyte-forming organs must be taken into consideration, an infectious or toxic breakdown of lymphocytes plays a principal part in the decrease of lymphocyte, and that there may exist a certain relationship between destruction of lymphocytes and formation of the large lymphocytes associated with lymphocytosis.

Miyagawa[15] has communicated a very interesting idea which is, "If the tissue or organ cells are parenterally injected into the organism, they act upon the homologous tissue or organ cells. The organ toxin responsed to the injected cells is generally created in the organism." Under his principle he and his coworkers have already published very many investigations on the mucous membrane of digestive tract, kidney, liver, lungs and so on. *Yokomori*[16] has found that the autolysate of lymphatic gland acted upon and degenerated lymphatic nodule, if it is parenterally inoculated into an animal. According to *Bayer*,[17] the product deliverated by stuff-exchange are resorbed into blood or lymph stream and may stimulate the other organ cells. *Isvesco*[18] proved experimentally that ovarian lipoid causes hypertrophy of ovary, lipoid obtained from red blood corpuscule urges an artificial anaemia to recovery, and lipoid obtained from thyroid gland act upon the same gland. Under the same principle, *Salkowski*[19] succeeded in performing the similar experiment. *Kapsemberg*[20] considered that the so-called leucotoxin produced by means of X-ray (Curschmann and Gaupp., Schmid and Geronne., Linser and Helber., Capp and Smith.) belongs not the true cytotoxin, but to

a certain toxic stuff freed from leucocytes, or to an intermediate stuff effected by X-ray. More recently *Naswitis*[21] has succeeded and also *Furukawa*,[22] to increase the red blood corpscule by means of administration of the same cells destroyed into animals. *Lichtwitz*[23] has employed such action of the red blood cells therapeutically and obtained an expected result.

From these literature as to the specific stimulation possessed by constituents of tissue or organ cells, and from my own investigations, it may be considered that bacterial toxin or any causative agent of a disease acts upon lymphocytes destructively, and the blood becomes laden with this abnormal product of lymphocytes, which has become soluble. It may also be considered that this abnormal disolved stuff produced by breakdawn of lymphocytes is an important factor for the appearance of the large lymphocyte with abundant protoplasm. Therefore, it is possible to establish a certain relationship between the foregoing stage of decrease of lymphocytes and the following appearance of the large lymphocytes. If my idea were not a mistake, similar changes of lymphocytes that are observed in the animals inoculated with typhoid vaccine, must be demonstrated by means of the inoculation of the products of lymphocytes destroyed artificially. Furthermore, the large lymphocytes must become demonstrable soon after the inoculation, because the products of lymphocytes destroyed are considered to stimulate the lymphatic tissues directly.

4. Influence of the preparation of lymphatic glands on blood lymphocytes.

A. First series of experiment.

Under such such expectation mentioned above, I have undertaken to examine what changes take place in the blood by means of administration of the autolysate of lympochytes intravenously into normal animals.

Material for injection was prepared from the mesenterial lymphatic glands from a normal rabbit. The glands were cut into small pieces and crushed, Thus numerous lymphocytes contained in milk-like fluid — chyle — were obtained. Such material was then mixed with 10 volums of destilled water and the mixture was allowed to autolyze in a test tube for about two hours at 37°C, and was filtered through ordinary filter paper. The liquid thus obtained is the preparation derived from lymphocytes which have been completely disintegreted by destilled water and I called it autolysate of lymphocytes. This preparation was deemed as a standard solution of disolved lymphocytes, and used in various dilutions if necessary. Whole procedures were of course carried out aseptically.

Dosage of administration was 0.5 cc. in total amount in each case, but the content of disolved lymphocytes varied.

Methods used in this study were the same as those in the puevious experiment.

Results obtained from the examinations of seven cases depended on the dose of administration.

a. Total number of leucocytes. Leucocytes were considerably diminished by the administration of a dose of 150 or 300 millions of disolved lymphocytes per kilogram of body weight. This leucopenia was caused chiefly by the decrease in the number of lymphocytes. It began immediately after the injection and continued for about one hour, and then was followed by leucocytosis which was largely referable to the polynuclear cells. The administration of a somewhat smaller dose of 30 or 50 millions of disolved lymphocytes per kilogram of body weight was followed by slight leucopenia and afterwards by leucocytosis. When a small dose of 10, 7 or 5 millions of disolved cells per kilogram of corporal weight was employed, leucocytosis occured in very slight degree from the beginning, or total number of leucocytes remained almost in a normal number. Generally speaking in this experiment, it is seen in all cases that the number of lymphocytes plays the principal part in the change of the total number of leucocytes. *Yokomori* has observed a similar symptom of leucocytic picture of circulating blood by injection of autolysate of lymph gland tissue into an animal.

b. The number of the lymphocytes with abundat protoplasm was markedly variable according to the dose injected. In the cases of injection of a small or somewhat larger dose they appeared numerously, amounting to several times more than normal number. Beside this, as it might have been expected, they showed sudden increase in a short time, that is from about fifteen to thirty minutes after the inoculation, and decreased rapidly again some four hours later, though the normal lymphocytes remained still almost in the same number. On the other hand when large dose were employed, they diminished or disappeared immediately after the inoculation, but became demonstrable some 24 hours later when normal lymyphocytes began to increase in number. Afterwards the blood formula resumed to its normal proportion.

In regard to the movement of lymphocytic picture a certain suitable dosage is an inevitable factor. A dose over the threshold inhibits the appearance of the large lymphocytes rather than stimulates.

B. Second series of experiments.

The preparation used in the former series of experiments in not a pure autolysate of lymphocytes but it is present in a mixture with chyle. I undertook therefore in this series of experiments to repeat the former examination in following manner.

The milk-like fluid which was obtained from mesenterial lymphatic glands was centrifugalized for about ten minutes at such a speed the majority of the cells were thrown down to the bottom of the tube, leaving a slightly clouded supernant fluid. The latter is pure chyle free from cells. The sediment was throughly washed with physiological salt solution and divided into two portion. One portion of them was mixed destilled water and allowed to autolyze. That

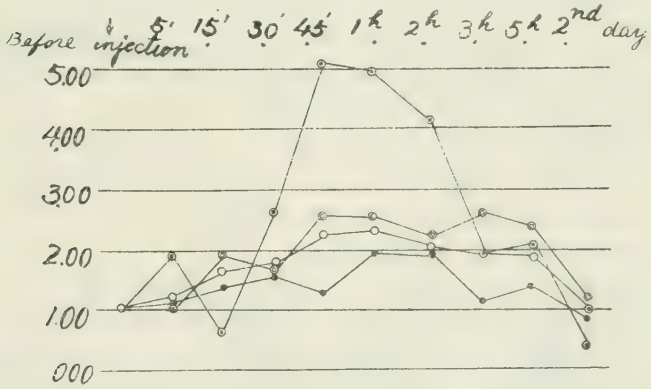
is relatively pure autolysate of lymph gland cells free from chyle. The other portion was suspended in physiological salt solution. The suspension contains the solid bodies of lymph gland cells in an inert fluid. These three liquids thus obtained, that is chyle, pure autolysate and suspension of lymph gland cells, were separately administered intravenously into three different normal rabbits in certain doses which is corresponded to their body weight.

Results obtained. I was able to obtain the same effects from the cases treated with the autolysate free from chyle as in the cases of the former series of experiments. For example, when a dose contained 10 millions of disolved lymphocytes per kilogram of body weight, it was recognized that the large lymphocytes with abundant protoplasm began to appear in thirty minutes after the inoculation, and diminshed about two hours later, though the other white blood cells showed not so apparent changes.

When animals were inoculated with the suspension of lymphocytes or with chyle in various doses, the blood formula of the each case was distinctly different in compare with the case treated with autolysate in a dose, which corresponded to that used in the former each case. In other words, in these cases I have never observed the increase of the large lymphocytes soon after the inoculation. These fact are illustrated in the following curve III, IV and V.

Curve III.

Leucocytic numerical change created by Injection with pure autolysate of 10 millions of lymphocytes per kilogram of body weight.



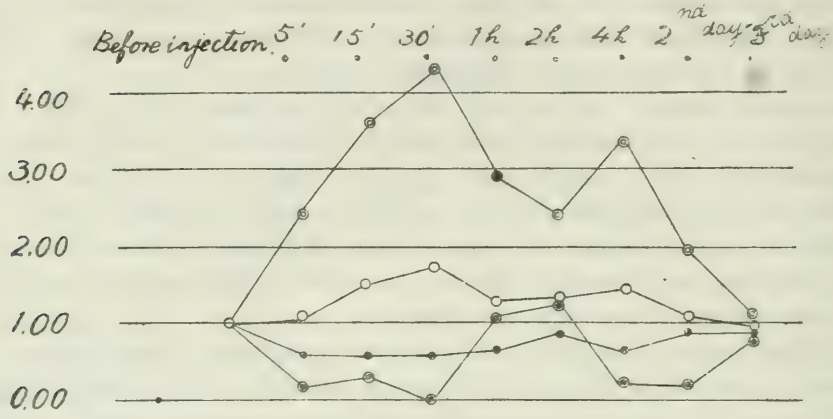
It is evident from these results that neither lymphocytes in itself nor chyle has an influence upon the appearance of the large lymphocyte with abundant protoplasm.

5. Influence of the autolysate of bone marrow and spleen cells on blood lymphocytes.

In addition to the foregoing experiments I undertook to employ autolysate of the cells of haemopoietic organ — bone marrow and spleen — as the material

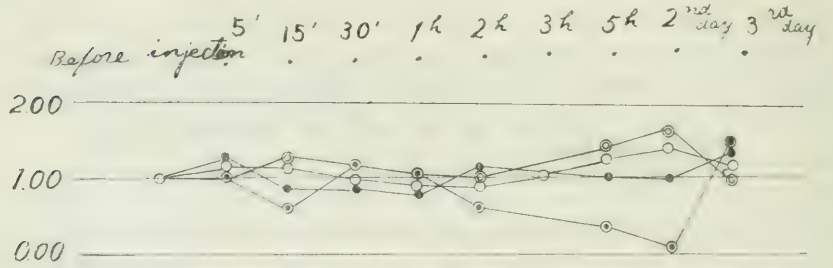
Curve IV.

Leucocytic numerical change created by Injection with suspension of 10 millions of lymphocytes per kilogram of body weight.



Curve V.

Leucocytic numerical change created by Injection with chyle free from cell of a dose in which 10 millions of lymphocytes were contained, per kilogram of body weight.



for inoculation. In spite of inoculation with various dosage of these preparations, blood change similar to the case injected with the autolysate of lymphocytes, could not be demonstrated.

It is established from these eperiments that though the autolysate of lymphocytes in a suitable dose promote directly the appearance of the large lymphocytes in the blood, neither uninjured lymphocytes nor autolysates of spleen and bone marrow cells have such power. If the latter, however, are administered in a so great dosage as to cause temporary leucopenia at the beginning, the large lymphocytes are aroused to appear numerously in the later course.

V. Summary.

1. The large lymphocyte whith abundant protoplasm which is found occasionally in the normal blood, become numerous under certain pathological conditions, such as mild cases of tuberculosis, typhoid fever in convalescence etc.

2. In the acute stage of infectious diseases when the decrease in the number of lymphocytes occurs, some pycnotic lymphocytes are observed. But they disappear in the late stage, and in this stage the large lymphocytes with abundant protoplasm become numerous associated with lymphocytosis. Such change in the number of various types of lymphocytes is seen especially in typhoid fever.

3. When typhoid vaccine is experimentally administered into a normal rabbit with such a dose which causes a diminution of lymphocytes in number, the lymphocytic formula of the animal shows a change similar to that of a patient suffering from typhoid feer.

4. From these facts mentioned above, it is highly probable that the lymphopenia in the early stage is always necessary to arouse a numerous appearance of the large lymphocytes in the later course, and also that the temporary lymphopenia at the beginning may be caused rather by means of the destruction of lymphocytes — which destruction is perhaps due to the infectious toxin —than by the lessened reactivity of the lymphatic tissues.

5. The substances freed from disintegrated lymphccytes are not normal to the blood and in consequence they must be in normal life-process of organism dissimilated and excreted as foreign bodies. It is, however, suggested if they exceed the normal degree in content in the blood, they may act upon the tissue or organ cells, and may especially cause a definite change of lymphocytes as Miyagawa stated. From such point of view, it is quite evident, that when I administered the autolysate of lymphocytes intravenously into a normal rabbit, the large lymphocyte appeared numerously in the blood soon after the inoculation, and diminished a short time later as I expected.

I must add here that in this experiment the dosage administered of the autolysate is of great importance. There is an optimal dose i. e. a threhold which stimulate promote the appearance of the large lymphocytes, if it is beyond, the inoculation is no effect.

VI. Conclusion.

It is concluded that the appearance of the large lymphocyte with abundant protoplasm is caused by the presence of destroyed substances of lymphocytes which have become soluble. Such substances are contained in very diluted concentration in the normal blood, so that they promote scarecely the appearance of such large lymphocytes in the blood circulation. But under certain pathological conditions, such as convalescence of acute infectious diseases or some mild chronic diseases, concentration of disintegrated lymphocytes in the blood become sufficient to stimulate the appearance of the large lymphocytes.

Contrary to this, the fact that the large lymphocyte is not demonstrable in the acute stage ef diseases may be explained by such a way that the destroyed

stuff of lymphocytes exists too much in the blood and therefore rather inhibits their appearance.

I close this article expressing my sincere gratitude for the kind guidance of Professor *Nagayo* and Professor *Miyagawa*, as well as the stimulation of Professor *Futaki*.

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Description of plate

1——16. Large lymphocytes with abundant protoplasm.

The nucleus of these cells is not circulare in outline and stain much less deeply than that of the usual small lymphocyte. One or more nucleoli can smoetimes be distinguished, appearing as clear translucent oval areas in the nucleus.

The cytoplasm forms a broad and extensive rim around the nucleus and stains a clear pale blue showing sometimes netlike structure. The outline is very irregular. The nuclear outline is often not well defined, and adjoining cytoplasm is less deeply stained than the periphery. A condition described as in the literature as the "perinuclear halo." A small or large number of azur granules in the cytoplasm can sometimes be made out.

17——21. Pycnotic lymphocytes.

The cell body is almost absent. The nucleus stains very deeply as a structureless mass and the outline is very irregular and fragmental.



(Dr. M. Okada : A Clinical and Experimental Study of Lymphocytes.)

Leukocytes and influenza.

By

Dr. **M. Okada.**

(From the Influenza Research Committee of the institute.)
(Publisched in the Japanese Journal of Experimental medicine
Vol. IV. October 1921.)

Many authors and investigators are mostly agreed with the typical pictures of leukocytes observed on many influenza patients, that simple influenza shows slight leukopenia and influenza pneumonia leukocytosis. Such leukocytic change is not yet recognized by all observers, especially from the standpoint of the numerical proportion of all kinds of leukocytes. I have examined the leukocytic change on many influenza patients, 64 cases, admitted in the institute. I was often surprised by the fact that the leukocytic pictures are sometimes perfectly differ from each other on the many patients in clinically nearly the same stage. What is the cause? If necessary, leukocytes counting was repeated many times in one case. From this investigation I am able to conclude that the number of leukocytes in influenza depends upon the stage and state of the patients.

My conventional classification of influenza.

From the stand-point of symptom of influenza and its course, it may be classified in many types. Some patients have only one cardinal symptom and others have two or three principal ones. Course of influenza is very irregular; some patients have very severe onset, but they recover after the lapse of few days, while others run prolonged courses and recover scarcely after 1 to 2 months. Contrary to these cases, the severest die within only few days duration from pneumonia which appeared from the beginning. From my own viewpoint, influenza is, therefore, divided into two groups; influenza simplex and influenza pneumonia.

Influenza simplex. I have called such cases influenza simplex, as they showed no distinct change on the bodily organs except only fever and catarrhal symptoms on the respiratory tract.

I. type; Slight fever and catarrhal symptom on respiratory tract. After the lapse of 3 to 4 days, the fever comes down to norm and the patients recover completely in a week after its onset. It is very slight case, therefore, the patients need not to be administered in hospital.

II. type; A little longer course than that of I type. Defeverescence takes place about in a week, but convalescence of disease durate more another one week. In this type I have observed often that the decrease of fever occured

once within few days and then the fever rose again suddenly. In such cases fever continues still more a week.

Influenza pneumonia. Pneumonia begins in various stage of influenza: (a) at the same time of influenza's outbreak, (b) in a few days after the onset of influenza or (c) after defeverescence of influenza. The courses of influenza pneumonia are not also the same in all cases and therefore according to the courses I have attempt to divide influenza pneumonia conventionally into 3 types, i. e. III. IV. and V.

III. type; At the onset and in first four or five days there is no difference from the influenza simplex, but after that pneumonic focus appears on the back lower portion of lungs. The defeverescence takes place usually in seven to ten days. Objective findings on lungs are seen pretty long time, in the majority of these cases in three or four weeks. In the acme of the disease, there is no frequency of breathing but about 20 to 25 per minute and no cyanosis on the lips, on the tips of fingers, nose and on other.

IV. type; The course of disease is more longer than that of III. type above described. The fever terminates usually in three to four weeks or more. The curve of fever shows irregurarity. The pneumonic foci are found not only on the back part of lungs but also on the anterior. The patients complain dyspnoea, with frequent breathing often 30 or more per minute.

V. type; This type is a little similar to the fourth type, but much severe. Pneumonic foci spread over the wide parts of lungs in a short time and consequently the patients complain cough and extreme dyspnoea with frequent breathing 40 to 50 or 60 per minute. Cyanosis occur on the cheek, nose, lips and fingers. The end of extremities become cold. The most patients are restless, delirious, sleepless and have no appetite for food. At the acme (about tenth day) of disease, the curve of body temperature, of pulse and breath come near each other on the fever chart. Some of patients die in this stage in spite of all medical and physical treatment. The others show quite slow decrease of fever and severe symptoms about two weeks after the onset, but still prolonged slight irregural fever in several weeks or about two months with pathological change on lungs. The above described conventional classification is applied in my investigation for the purpose to detect some differences in each type and stage of influenza.

Picture of leukocytes.

I have examined the number of total leukocytes and counted differencially the each sort of them on the stained films. But no characteristic change for influenza was observed except the relationship between the number of N. (neutrophile polynuclear leukocytes) and L. (lymphocytes). I will, therefore, show only the number of total leukocytes, N. and L. in the following tables.

I. type; 3–4 days duration of fever and completely recovered in a week.

Patient	Age & sex	Febrile duration	Duration of disease	Examining day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.
O, K.	19♀	3	5	2nd	8.200	50	4.100	45	3.700
S, T.	24	4	7	4th	6.800	45	3.100	40	2.700
S. T,	24♂	4	7	4nd	9.400	47	4.400	47	4.400
				12th	7.000	45	3.200	45	3.400
K, M.	23♂	3	5	2nd	7.000	60	4.200	39	2.700

II. type; 6–7 days duration of fever and recovered in 10–14 days.

Patient	Age & sex	Febrile duration	Duration of disease	Examining day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.
K, Y.	43♂	6	10	3rd	5.000	37	1.900	58	2.900
				10th	6.000	46	2.800	49	2.900
M, Y.	18♂	6	11	3rd	5.000	50	2.500	49	2.500
				6th	4.400	26	1.200	72	3.200
				9th	5.000	21	1.500	76	3.800
S, T.	19♂	6	11	5th	6.200	70	4.300	28	1.700
				12th	7.200	42	2.300	54	3.900
H, R.	20♀	7	12	4th	4.600	70	3.200	23	1.100
				10th	5.000	54	2.700	43	2.200
N, M.	17♀	7	13	5th	4.800	68	4.300	26	1.300
				12th	7.500	61	4.600	26	2.000
J, G.	13♀	7	13	4th	4.800	55	2.600	36	1.700
				17th	9.000	60	5.400	35	3.200
H, S.	25♀	8	13	7th	4.000	71	2.800	25	1.000
				13th	6.000	63	3.800	30	1.800
K, D.	30♂	7	14	7th	6.800	54	3.700	39	2.700
S, Y.	20♀	7	13	6th	10.500	83	8.700	14	1.500
				12th	11.000	74	8.100	17	1.900
W, N.	38♂	7	12	4th	15.000	86	13.000	6	900
				9th	10.000	61	6.100	28	2.800
S, T.	27♂	16	21	8th	8.000	55	4.400	42	3.400
				16th	15.000	86	13.000	12	1.800

III. type; about 7–10 days duration of fever and recovered in 2 or 3 weeks.

Patient	Age & sex	Febrile duration	Duration of disease	Examining day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.	Day of appearance of pneumonia
C, S.	41♀	6	16	3rd	5.000	47	2.400	39	1.800	10th
				7th	6.000	44	2.600	41	2.500	
				11th	7.400	53	3.900	43	3.200	
S, K.	24♀	6	14	7th	5.000	47	2.400	47	2.400	2nd
S, T.	23♀	6	15	5th	5.500	63	3.500	34	1.900	5th
				10th	6.000	52	3.100	40	2.400	
O, G.	18♀	7	16	5th	5.500	52	3.000	44	2.400	5th
				10th	4.500	52	7.300	34	1.400	
K, N.	38♂	7	14	6th	5.800	70	4.100	26	1.500	7th
				9th	5.600	57	3.200	34	1.900	
				13th	5.000	52	2.600	36	1.800	
I, K.	30♀	7	14	5th	6.000	57	3.400	41	2.500	3th
				12th	9.000	65	5.900	27	2.400	
A, Y.	24♀	7	14	4th	6.000	63	3.800	36	2.200	2nd
T, S.	23♀	7	14	4th	5.800	67	3.900	31	2.000	2nd
				13th	11.000	64	7.000	30	3.300	
C, J.	29♂	8	20	7th	4.500	37	1.700	60	2.700	5th
				16th	6.000	78	4.700	20	1.300	
W, B.	28♂	9	16	8th	4.600	41	1.900	57	2.600	3rd
O, D.	28♂	9	19	3th	9.000	53	4.800	44	4.000	2nd
				12th	7.600	63	4.800	36	2.700	
				17th	7.600	67	5.100	28	2.100	
M, W.	48♂	9	21	7th	10.000	50	5.000	49	4.900	7th
				10th	9.000	70	6.300	27	2.400	
				14th	9.000	84	7.600	10	0.900	
				18th	8.000	82	6.600	16	1.300	
				24th	7.500	61	4.300	36	2.700	
T, K.	45♂	9	21	6th	4.000	55	2.200	37	1.600	4th
				15th	10.400	80	8.300	18	1.900	
Y, G.	29♂	9	23	8th	4.500	59	2.700	36	1.800	2nd
				18th	9.000	57	5.100	43	3.700	
M, M.	16♂	10	24	5th	7.800	40	3.900	43	3.400	14th
				12th	8.000	44	3.500	55	4.400	
				15th	9.600	65	6.200	33	3.200	
				20th	7.600	50	3.800	42	3.200	

N, J.	20♂	10	32	8th	5.500	60	3.300	36	2.000	3rd
				24th	11.000	72	8.000	25	2.800	
H, D.	24♀	10	17	14th	8.800	59	5.200	32	2.800	8th
H, T.	19♀	11	20	10th	5.000	73	3.700	24	1.200	4th
				17th	4.200	42	1.800	52	2.200	
S, T.	22♂	11	16	11th	6.800	33	5.500	63	4.300	8th
				14th	13.000	40	5.200	59	7.700	
H, B.	21♂	11	23	22th	4.900	47	2.100	52	2.600	13th
N, Y.	21♀	11	22	11th	6.500	82	5.300	14	900	4th
				16th	9.400	76	7.100	20	1.900	
T, M.	57♂	8	14	7th	12.000	82	10.000	15	1.800	3rd
				11th	11.400	69	7.900	21	2.400	
S, S.	25♂	9	19	8th	11.500	82	9.400	12	1.400	3rd
I, G.	37♂	9	19	8th	15.000	73	11.000	20	3.000	7th
				18th	9.000	39	3.500	55	5.000	
K, S.	19♂	10	20	9th	10.000	80	8.000	17	1.700	8th
T, M.	59♂	10	18	5th	9.200	90	8.300	6	800	6th
				15th	9.000	73	6.600	26	2.300	
T, B.	40♂	11	20	10th	11.000	83	9.100	14	1.500	3rd
				16th	7.000	69	4.800	28	2.000	
I, U.	23♂	15	23	8th	7.000	65	4.600	29	2.000	5th
				17th	6.000	75	4.500	18	1.100	

IV. type; Prolonged cases of imfluenza pneumonia.

Patient	Age & sex	Febrile duration	Duration of disease	Examining day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.	Day of appearance of pneumonia
S, O.	65♀	25		3th	14.000	80	12.000	16	2.300	3rd
				5th	17.000	71	12.000	22	3.700	
				10th	22.500	86	19.000	10	2.300	
				18th	18.000	63	11.000	31	5.600	
N, Y.	37♂	34		27th	10.800	83	9.000	12	1.300	5th
				31th	8.600	60	5.200	31	2.800	
M, N.	27♀	23	32	18th	17.900	91	16.300	9	1.600	10th
				22th	12.400	84	10.400	13	1.600	
				32th	9.200	57	5.200	39	2.600	

N, T.	49 ♂	23	42	4th	8.000	76	6.100	22	1.800	1
				9th	12.000	85	10.200	12	1.400	
				21th	10.000	77	7.700	21	2.100	
				34th	8.000	53	4.200	41	3.300	
H, G.	22 ♂	27	37	8th	11.400	80	9.100	16	1.800	2nd
				12th	14.800	70	10.400	23	3.400	
				16th	9.400	69	6.400	24	2.300	
				23th	11.000	61	6.700	31	3.400	

V. type; (a) Severe cases of influenza pneumonia.

Patient	Age & sex	Febrile duration	Duration of disease	Examining day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.	Day of appearance of pneumonia
N, S.	20 ♂	13	25	11th	10.000	78	7.800	20	2.000	3rd
				16th	9.600	65	6.200	30	2.900	
				24th	7.500	53	4.000	42	3.200	
M, R.	30 ♀	33	40	5th	9.200	77	7.100	21	2.000	7th
				9th	16.000	86	13.800	12	1.900	
				15th	16.000	80	13.000	15	2.400	
				27th	12.000	72	8.600	21	2.600	
O, T.	38 ♂	23	32	9th	11.000	85	9.400	19	1.100	1st
				16th	15.000	77	11.600	17	2.600	
				23th	15.000	79	11.900	19	2.900	
N, S.	42 ♀	32	53	14th	18.000	95	17.100	3	500	
				19th	20.000	92	18.400	6	1.200	
				24th	12.600	86	10.800	13	1.600	
				28th	13.000	78	10.100	20	2.600	
				33th	8.000	70	5.600	25	2.000	
				53th	15.000	69	10.400	35	3.300	
N, G.	25 ♀	16	39	11th	10.000	80	8.000	16	1.600	3rd
H, I.	38 ♂	19	36	19th	15.000	17	10.700	20	3.900	4th

V. type; (b) dead case from influenza pneumonia.

Patient	Age & sex	Day of death	Examined day	Number of total leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.	Day of appearance of pneumonia
T, M.	36 ♀	10	10th	11.000	—	—	—	—	10th
M, H.	28 ♀	11	5th	10.000	81	8.100	16	1.600	9th
G, D.	28 ♀	11	9th	13.000	83	10.800	15	2.000	2nd

T, M.	46♀	11	2nd	11.500	83	9.400	13	1.500	2nd
			10th	50.200	94	47.300	4	8.000	
O, D.	19♀	12	10th	10.000	—	—	—	—	2nd
A, S.	19♀	12	10th	14.600	88	12.800	8	1.300	3rd
M, O.	45♀	12	2nd	8.000	86	6.900	10	800	4th
			7th	15.000	79	11.900	19	2.900	
			11th	20.000	81	24.300	12	3.600	
O, K.	19♀	12	5th	8.000	73	5.300	24	1.900	4th
			10th	17.000	85	14.500	13	2.200	
K, J.	41♀	17	6th	7.500	64	4.800	34	2.600	1st
K, M.	27♀	20	8th	18.000	82	14.800	14	2.500	1st
S, T.	46♀	25	5th	31.100	92	28.600	4	1.200	1st
			12th	33.000	91	30.000	7	2.300	
			17th	26.000	88	22.900	8	2.100	

Summary and conclusion.

The total number of leukocytes in normal individual is generally accepted to amount to 6.000—10.000, absolute number of N 5.000—5.500 and L's one 1.500—2.000. Comparing with these normal standard, I will summarize the result obtained.

1) At the beginning of disease there is no remarkable change in the number of total leukocytes in each type of influenza. But the numerical proportion of N and L is various in different type; In slight cases decrease of N and increase of L in number are observed. In severe cases of pneumonia the numerical proportion of N and L is almost normal or there is slight increase of N and slight decrease of L in number.

2) The changes of leukocytic picture in acme of disease are summarized in the following table.

	Total number of leukocyte	N. %	Absolute number of N.	L. %	Absolute number of L.
I type	non-decrease	decrease	decrease	increase	increase
II type	slight decrease	decrease	decrease	slight increase	increase
III type	slight decrease	decrease	decrease	slight increase	increase or norm
IV type	increase	increase	increase	decrease	norm or increase
V type	increase	increase	increase	decrease	norm or increase

3) The changes of leukocytic picture in convalescence are showed in following table.

	Total number of leukocytes	N. %	Absolute number of N.	L. %	Absolute number of L.
I type	norm	decrease	decrease	increase	increase
II type	norm or increase	slight decrease	slight decrease	increase	increase
III type	norm or increase	decrease or norm	decrease or norm	increase	increase
IV type	increase	norm or increase	norm or increase	norm or increase	increase
II type	increase	increase	increase	norm	increase

4) I am able to conclude from the above described results the following fact. In the slightest influenza there is no change in total number of leukocytes but increase of L in number. In moderate cases, without regard to influenza simplex and influenza pneumonia, there is seen in acme of the disease a slight leukopenia and lymphocytes become numerous first in this stage. In severe pneumonia the total number of leukocytes increase in number in acme and do not decrease even in convalescence and lymphocytosis is seen finally in convalescence.

5) From the above mentioned leukocytic picture I am able to believe that the prognosis of influenza may be determined by the counting of total number of leucocytes and by the differential counting on the stained films.

Morbid anatomy of influenza during 1918-1920.

By

Prof. Dr. **Kiyoshi Sato.**

(From the Influenza Research Committee of the Institute.)

The author has examined the pathological anatomy in thirty-four cases of influenza and the principal findings were as follows:

1) The majority of morbid cases were juvenile persons aged 20–30 years, agreed with the statistics by european investigators. I could not find any bodily disposition in such young persons, so suffering from influenza for example, status thymico-lymphaticus, stenosis of the aorta or any other anomalous bodily development.

The cases with pneumonia were adopted in author's examination. They showed various appearances depending upon the clinical course of grippe and the individuality of the reaction on the whole organs.

The typical form is a broncho-pneumonia accompanied in the lung tissue serous and hemorrhagic infiltration, i. e. the hemorrhagic broncho-pneumonia, having 50% in all cases, agreeing with French's, Lyon's, Opie's, Treemann's, Blake's, Small's, River's, Yamagiwa's, Fujinami's, Kawamura's and Mitamura's descriptions. Beside this, there are confluensed catarrhal pneumonia (30%), supprative broncho-pneumonia (70%), interstitial (3%), lober catarrhal fibrinous (7%) and only in one case croupous pneumonia.

2) I found a certain relationship between pneumonic focus and bacteriological findings, namely the Pfeiffer's bacilli are deemed to be found in the pustulous focus of capillary bronchus in 64,6% among all cases, especially abundance in the cases of hemorrhagic broncho-pneumonia, pneumococci in 94%, diplococcus catarrhalis in 60% and streptococcus in 41%. Beside these I found pretty small unknown fuchsinophilic granules in the lung tissue. The above mentioned bacteriological findings are similar to the description of MacCollum, French, Lucke, Wright, Kime, Keegan, Opie, Treemann, Blake, Small and Fujinami. Pleurisy consisting of hypermia, diapedesis and fibrinous investment, was found not seldom and seemed to be produced as a secondary complication of pneumonia as the description of Oberndorfer, Simmonds, Dietrich, Yamagiwa and Fujinami. Suppurative pleurisy is complicated with the lobar fibrinous-catarrhal pneumonia.

3) The pathological finding of heart muscles is the same with the description of Stone and Swift. The main points consist of the dilatation of the right chamber

and turbidity of muscles, rarely its necrosis and very seldom acute myocarditis.

4) Blood vessels. In the coronal artery the elastica interna is dissoluted and often brocken up in a few part and in the media a slight edema was seen in 2 cases. Pretty large thrombus in vena cava inferior from a left adrenal vein was observed.

5) Nerve System. In one case I found a encephalomeningitis caused by streptococci, the latter being cultivated from those foci.

This inflammation was considered as a serous encephalomeningitis, differing from the so-called encephalitis lethargica.

6) Stomach and intestine. In the majority of cases there are small hemorrhagic spots and catarrh on the mucous membrane. In a few cases slight enlargement of lymph-follicles in jejunum.

7) Liver. In the liver principal changes were, as described by Luke, Wright, Kime, Yamagiwa and Fujinami, turbidity, swelling and hyperemia in parenchym, rarely fatty metamorphosis, often slight interstitial inflammation.

8) Spleen. Splenic enlargement was always very slight or absent never seen such as infectious spleen, just like that of the description Abrahamus, Hallaws and French. The lymph-follicles showed atrophy and are often encountered regressive changes in its centre. The sinus were dilatated and the pulpa cells diminuted in number as described by MacCollum, Nolou, Lewis, Washington as well as by Yamagiwa.

9) Lymph nodes. Those situated in the lung hilus were affected always very severely and are enlarged showing hemorrhage.

They were more seriously changed in below than above the bifurcatio tracheae. These lesions are much more pronounced in lymph nodes below the bifurcatio than above.

10) Bone marrow. Necrotic foci are rarely met with.

11) Adrenals. The lipoid contents in cortical sucstance were generally diminished and adrenalin seemed to be reduced.

12) Muscles. Zenker's degeneration was very often seen in abdominal right muscles and in uterus necrotic change was sometimes observed.

13) Kidneys. In kidneys there were seen turbidity, swelling and hyperemia in parenchym and very seldom fatty metamorphosis and necrosis.

In serous membrane there were seen very often hemorrhagic patches, but no change on the skin.

From the pathological and anatomical point of view, the changes of whole bodily organs are very multifarious and on the essential points they are agreed with the description made by *MacCollum Lucke, Wihtg, Kime, Keegan, Opie, Treemann, Blake, Sweil, Yamagiwa* and *Fujinami*.

Experimentelle Studien über die Pathogenese der Amyloidose.

Von

Prof. Dr. **Kiyoshi Sato.**

(Aus dem Pathologischen Abteilunge des Institutes,
Vorstand. Prof. Mataro Nagayo.)

Um die Genese des Amyloids histologisch zu studieren hat der Verf. bei Kaninchen, Ratten, Mäusen und Tauben subcutane Injectionen von verschiedenen Eitererregern, vorwiegend Staphylococcus aureus vorgenommen, wobei hauptsächlich bei Mäusen verschiedene Versuche stattfanden, und hat er die seit 1921 erzielten Resultate, wie folgt, kurz zusammengefasst:

1) Der Ausfall der Amyloidbildung bei den Tieren beruht auf der Menge der injecierten avirrulenten lebenden Bacterien, aber der Verf. konnte durch Injection der Filtrate und sterilisierten Kulturen derselben bei Mäusen Amyloid nicht konstatieren.

2) Die Amyloidentartung bei Mäusen wird zuerst in der Milz gefunden, wobei formalgenetisch diese Substanz zu den Lymphfollikeln in inniger Beziehung stand. Dabei waren im Zelleibe der Lymphocyten, der Plasmazellen und der Grossemononucläere in der peripheren Teilen derselben metachromatisch verfärbte und mit Fetttropfen vermischte Amyloidtropfen zu finden, deshalb möchte der Verf. solche Veränderung der Lymphfollikel als frühestes Stadium der Amyloidentartung bezeichnen, wobei dann durch Zerfall solcher Zellen und durch eine physicochemische Verbindung mit Gewebssaft eine gelöste Amyloidsubstanz, bezw. eine hyaline Substanz im weiteren Sinne, welche sich an den Reticulumfasern oder Reticulo-endothelien abgelagert, im Betracht kommt.

Der Verf. möchte diesen Vorgang als ein zweizeitiges Stadium der Amyloidentartung bezeichnen, da er ausser der fettigen Degeneration der Zellelemente auch noch eine Infiltration der Lipoidsubstanz wahrgenommen hat.

3) Bei den mit der 70% Alkohol-fixierung des Materials und mit den Gefrierschnitten behandelten Praeparaten hat der Verf. nachgewiesen, dass das sog. jungendliche Amyloid des frühesten und zweizeitigen Stadiums nicht eine achromatische, wie gewöhnliches Hyalin, sondern von Anfang an eine metachromatische Substanz ohne Jod- oder Jodschwefelsäurereaction ist. Der Verf. injizierte wässerige Cholesterinemulsion in die mit Amyloid versehene Milz und in den 2 Wochen nach der Injektion trat die Jod- und Jodschwefelsäurereaction mässig deutlich hervor.

Wenn man ein jungendliches Amyloid mit dem Grossnetz eines anderen

Tieres in Verbindung stehen lässt, erscheint etwa 24 Stunden darnach die Jodreaction mässig deutlich, die Jodschwefelsäurereaktion jedoch fast gar nicht.

Von oben erwähnten Vesuchen ausgehend möchte der Verf. annehmen, dass eine chemische Veränderung der Cholesterinhaltigen Substanz in Amyloidentartungsherden höchstwahrscheinlich für die grünnliche Jodschwefelsäurereaktion, welche eine eigentliche, reife Reaktion des Amyloids zeigt, eine Rolle spielt.

4) In Bezüg auf die Mobilisation, bezw. Infiltration der Amyloidsubstanz aus dem primären Organ in andere durch das Gefässsystem hat der Verf. kein positives Resultat bekommen.

5) Um die Amyloidentartung beim Tiere, besonders bei Mäusen hervorzugerufen hat der Verf. als eine begünstige Vorbedingung eine Hyperplasie der lymphatischen Gewebe gefunden, nämlich bei Mäusen, an welchen die Lecithin oder Fermentinjektion, wie Pankreatin, Tripsin, und die Lipoidfütterung, wie Butter-Brot gemacht worden sind, waren mässige Hyperplasie der Milzfollikel und Amyloidentartung zu sehen, aber der Verf. konnte noch nicht eine nur durch Fermentinjektion hervorgerufene Amyloidose konstatieren.

6) Beim Mäusekrebs hat der Verf. noch nicht spontane Amyloidose gefunden, aber wenn man mehrmals die Injektion von einer Salzsäure-pepsinlösung in die Krebsherde vornimmt, kann in kurzer Zeit Amyloidbildung in der Milz hervorgerufen werden.

7) Von oben erwähnter Thatsache ausgehend möchte der Verf. annehmen, dass die beim Menschen beobachteten Amyloidentartungen, die in vielen Literaturen angegeben ist, als eine Vorbedingung derselben, zu den besonderen Veränderungen der lymphatischen Gewebe, d.h. zu den Hyperplasien in inniger Beziehung steht.

8) Bei Fütterung mit Knorpelpulver oder der subcutanen Injektion der Knorpelemulsion konnte der Verf. keine Amyloidose konstatieren.

9) Um den Nahrungseinfluss auf den Ausfall der Amyloidentartung zu studieren hat der Verf. Fleisch-, Reis- und Eidotterfütterung bei Mäusen vorgenommen, hat aber ausser bei der Butter-Brotfütterung kein besonderes Resultat bekommen.

10) Bei den Milzexstirpierten Mäusen konnte der Verf. auch Amyloidose konstatieren, wobei eine wichtige Bedingung ist, dass die Lymphocytenanhäufungen. bezw. Lymphfollikelähnliche Gebilde in der Leber, der Niere und anderen Organen erscheinen. Daher möchte der Verf. behaupten, dass locales Amyloid und allgemeine Amyloidose genetisch ganz gleich sind.

11) Die einmal entstandene sog. jungendliche Amyloidsubstanz im Gewebe kann spontan oder durch die entzündlich infiltrierten Zellen, wie Leucocyten, Histiocyten absorbiert werden, wobei die Fremdkörperriesenzellen in der betreffenden Milz hauptsächlich als Heilungsreaktion reichlich vorhanden sind.

(*Autoreferat.*)

Über die Leberveränderung bei Adrenalinvergiftung.

Von

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Um die durch Adrenalininjektion hervorgerufene Leberveränderung, insbesondere die Frage, ob eine Lebercirrhose zu stande kommt oder nicht, zu studieren hat der Verf. bei Kaninchen intravenöse Injektion von Adrenalinlösung vorgenommen. Der Verf. hat an 2.8–2.5 kg. schweren Kaninchen nach 1 bis 2 maligen Injektionen von 1,–2. cc. 1.% Adrenalinlösung akute Vergiftungsveränderungen von Hyperaemien, Blutungen und Nekrosen in der Leber und anderen parenchymatösen Organen gefunden. Bei den Tieren nach 12–41 maligen Injektionen von kleiner Menge dieser Lösung in 20–53 Tagen konnte der Verf. eine ausgesprochene cirrhotische Veränderung in der Leber konstatieren; dieselben besitzen nämlich makroskopisch eine Volumabnahme, eine unebene, granulierte Oberfläche und eine zunehmende Konsistenz. Bei der mikroskopischen Untersuchung waren ausgesprochene Neubidungen von Bindegewebe zu sehen, welche an den Pfortaderästen in Glissonschen Scheiden entlang auffallen, sowie eine geringe Veränderung der Leberzellen, welche meist atrophisch geworden sind, weshalb die ganze Beschaffenheit dieser Leber an die Läennecsche Lebercirrhose erinnert.

Aus den experimentellen Untersuchungen möchte der Verf. das Vorhandensein der primär durch entzündliche Wucherung der interlobulären Bindegewebe entstandenen Lebercirrhose annehmen, jedoch war dieselbe bei chronischer Adrenalinvergiftung nicht immer nachgewiesen, so dass für den Ausfall der cirrhotischen Veränderung im diesen Organe die Art und die individuelle Dispostion der Tiere eine grosse Rolle spielt. Was den aetiologischen Moment des durch Adrenalininjektion hervorgerufenen cirrhotischen Vorganges betrifft, so möchte der Verf. hauptsächlich einen Reiz von eigentlichem Adrenalingift annehmen, ob dabei die Blutdrucksteigerungstätigkeit derselben in irgend welcher Beziehung dazu steht oder nicht, bleibt von geringer Bedeutung.

(*K. Sato.*)

On the Pathology and Pathologic Anatomy of Experimental Acidosis.

By

Dr. **Tohru Satow.**

(From the pathological Department of the Institute; Director of the Department Prof. Mataro Nagayo.)

Acidosis was artificially produced in hen and rabbit by giving hydrochloric acid through mouth. The condition of acidosis was ascertained by the amount of carbonic acid gas in the blood estimated by means of Van Slyk's vacum apparatus and titrating method. For urinanalysis the specimen was furnished mostly by rabbit while for histological study hen was used with preferrence. Haemocytology, haemoanalysis and body weight were studied in both animal seris. Control examinations were carefully carried out on the hunger condition of animals because this is very closely related to acidosis.

The result of the study can be summerized as follow:

According to the decrease of carbonic acid gas in the blood suger (Epstein's microsacchrometer used) in the plasma would increase while the body weight reduces as a rule. With increase of the blood sugar albumen will be found in the urine and when the blood sugar attains its maximum reducing substance will make its appearance in the urine.

The weight of important organs were compared with that of control animals in the relative weight; of the organ divided by weight. The relative weight of kidney and adrenal shows considerable increase in experimented animals as well as in hunger specimen, the former being more marked than in the latter.

In the case of acidosis lymphocitic cells accumulate in cardiac muscles, periportal cells accumulate in liver, follicles of spleen and bone marrow presents a notable atrophy. Bony tissue shows an indication of calcium decrease both macro- and microscopically. Contrary to the statement of Householder the writer is unable to find splenic tumor but is able to find pigmentous deposit which has possibly derived from haemoglobin. Fatty substance taking sudan III stain is observed around the athophic follicles and inside of the reciculum cells.

Parenchyma cells of liver in acidosis are void of glycogen with earlier onset than in hunger cases and the vacuole formation would take place more markedly in the former than in the latter. Lipoid substance in parenchyma of liver increases considerably assuming globular appearance. The writer has encountered no cases in which Mosse's reaction has proved to be decidedly positive. Ewing remarks

that the gall bladder distends in cases of acidosis but this is probably due to hunger as has been shown by the writer's statistical survey.

Kidney epithels manifest picture of cloudy swelling accompanied by vacuole formation and increase of fatty substance. Abraision of zona peniculi is also of constant occurrence.

Adrenal would show invariably vacuole degeneration, this being probably related to the increase of the weight, for other alterations are of minute importance.

Peripheral nerves were stained by Cajal's, Bielschowsky's and Marchi's method. Axis cylinders stain irregularly, pale and dark stained portions alternating in patchy manner. The nerve fibre are extremely irregular in thickness, narrow thread-like part, flattened band-like portion and spindle formed swelling intermingling. The balooned part stains generally pale, some showing losened neurofibrillary structure while others devoid of any contents except some glassy homogeneous substance. The swelling may sometimes contain vacuoles in which nothing can be demonstrated even by frozen section method. Myelin sheaths also present segmentary swelling in which axis cylinders present themselves in spiral or whorl-like manner and even as amorphous detritus mass. The latter takes haematoxylin stain which the normal axis cylinder never does. This condition be of artefact since the same is occasionally encoutered in control specimen though in milder grade. However this may be considered none the significant because so conspicuous a finding is never to be found in other conditions.

Myelin sheaths take osmic acid decidedly deeper than in control, thus obscuring the fine architecture of the seaths. Granules stainable with osmic acid increase in number and vacuoles may often be encountered in the sheaths.

Beiträge zur Kenntnis der biologischen Wirkung des Chlornatriums auf das Blut und die hämatopoetischen Organe

Von

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Vorstand Professor Dr. med. Mataro Nagayo.

(In der japanischen Sprache veröffentlicht in "The Japanese Journal of Experimental Medicine" Vol V, No. 6. Febr, 1922. und vorgetragen im medizinischen Verein dieses Instituts am 2. Dezember 1922.)

Hierzu Tafal 1.

Über die biologische Wirkung des Kochsalzes ist schon mehrfach berichtet worden. Man kann diese von vier Seiten aus betrachten.

A. Das Kochsalz ist als Bestandteil der Nahrungsstoffe ein wichtiges Kapitel der Diätetik. Und auf dem Gebiete des Stoffwechsels ist das Kochsalz ein Hauptgegenstand physiologisch-chemischer Untersuchungen.

B. Zweitens muss man die Studien über die physikalisch-chemischen Wirkungen des Kochsalzes auf die Organismen erwähnen. Das Kochsalz macht wohl einen wichtigen Bestandteil der osmotisch wirksamen Substanz der Körpersäfte aus. Es spielt eine wichtige Rolle bei den Lebenserscheinungen der Organismen, weil die Kolloidalzustandsänderung hauptsächlich von der Verteilung der Salzionen abhängt. In Bezug auf Osmose übt das Kochsalz durch den Umtausch seiner Moleküle und Ionen eine gewisse Energie auf die Organismen aus. Den in Blut und Gewebssäften gelösten anorganischen Salzen fällt die Aufgabe zu, den osmotischen Druck in den Zellen und damit die Funktion des Organismus nach allen Richtungen hin konstant zu erhalten. Besonders hat das Kochsalz, wesentlich gelöst in Blutplasma, eine wichtige Funktion bei der Regulation des osmotischen Drucks zwischen Zellen und Säften, sowie bei der Gleichgewichtshaltung der Blutreaktion.

Die freie Salzsäure im Magensaft entsteht nach *Köppe* direkt durch den Ionenaustausch zwischen H-Ion aus dem Blute und Cl-Ion aus dem Kochsalz im Magenlumen. Für die Entstehung des Odems macht die Kochsalzretention mit der Veränderung der Gefässwand und der Gewebe einen wichtigen Faktor aus. *Von den Velden* erklärte die blutstillende Wirkung der Injektion konzentrierter Kochsalzlösung dadurch, dass die Mobilisierung der Thrombokinase in das Blut aus dem Gewebssaft infolge der Veränderung der Konzentration stattfindet. Be-

sonders sei hier erwähnt, dass *Hugo de Vries* 1882 durch Studien an den Zellen der Blattepidermis von Tradescantia discolor die Erscheinung der Plasmolyse erkannte und über „Isontenie, Hypo- und Hyperisotonie" schrieb. 1883 machte *Hamburger* die gleichen Studien an den menschlichen Erythrozyten. Die isotonische Kochsalzlösung wird als ein indifferentes Medium in der Medizin sowie im Experiment allgemein gebraucht.

C. Spezifische Wirkung oder Ionenwirkung des Kochsalzes. S. *Ringer* hat den schädlichen Einfluss der reinen 0.75%igen Kochsalzlösung auf das ausgeschnittene Froschherz gefunden und der Kochsalzlösung Kalzium- und Kaliumsalze als Antagonisten gegen das Kochsalz zugesetzt.

Einer besonderen Erwähnung bedürfen hier die wichtigen Untersuchungen J. *Loebs* über die Bedeutung der Ionen für die Entwickelung der tierischen Zellen und Lebewesen. Er hat nämlich bewiesen, durch die berühmte Untersuchung an Fundulus-Eiern, dass das Kochsalz in einer Lösung von bestimmter Konzentration die Entwicklung dieser Eier aufhebt, Zusatz von Calciumsalzen jeder Art regt sie wieder an. Dagegen erweisen sich die Alkalisalze als unwirksam. Es handelt sich hier um Giftwirkung und Entgiftung. Andererseits ist nach *Overton* die Gegenwart von Na-Ion nicht nur für den Kontraktionsakt sondern auch für die Energieleitung durch die Muskelsubstanz erforderlich. 1869 hat *Cohnheim* zuerst die intravenöse Injektion von Kochsalzlösung ausgeführt. *Cohnheim* und *Lichtheim* haben den Kaninchen in die Halsvenen eine kolossale Menge von 0.6%iger Kochsalzlösung eingeführt. Die Kaninchen gingen nach 2 Stunden zugrunde, und wurden dabei durch die Sektion Ödem an den Eingeweiden, Speichel- und Tränendrüsen gefunden, dagegen blieben die Thoraxhöhle und subkutanes Gewebe auch nach massenhafter Infusion ausnahmslos völlig trocken. *Ph. Knoll* und *Albu* haben gerade die umgekehrten Resultate an dem Unterhautgewebe von Kaninchen bekommen. *Magnus* hat bewiesen, dass die Kochsalzinfusion kein allgemeines Hautödem an Kaninchen ohne Gefässwandschädigung hervorruft.

D. In Bezug auf die Organveränderung durch Kochsalzinfusion haben *Hess* fettige Degeneration an der Leber und leichte trüber Schwellung an der Niere, *Wideröe* kleine Blutung und parenchymatöse Veränderung am Herzen, *Raum* die hydropische Durchtränkung und Vakuolisierung der Leberzellen, *Achard* und *Paisseau* hochgradige Entartung der Nierenepithelien beobachtet. *Leopold* hat durch orale Darreichung von kleiner Menge Kochsalz parenchymatöse Veränderung der Niere und Albuminurie beobachtet. *R. Matsuyama* hat an Kaninchen und Ratten durch wiederholte Injektion von grosser Menge Kochsalzlösung Dilatation und leichte fettige Degeneration des Herzens, leichte fettige Degeneration der Leber, parenchymatöse Entartung und Hyperämie der Niere und Hämosiderinablagerung in der Milz konstatiert.

L. *Goldbach* hat an Menschen innerhalb 2 Stunden nach Injektion von kleinen Mengen physiologischer Kochsalzlösung leichte Verminderung der Erythrozyten und eine etwa doppelte Vermehrung der Leukozyten beobachtet. Dabei

zeigte es sich, dass nicht die Lymphozyten vermehrt waren, sondern die polynukleären Leukozyten mit neutrophiler Granulation. *Miyake* fand gleich wie *Issajeff*, dass nach einer intraperitonealen Injektion von Kochsalzlösung eine Periode der Hyperleukozytose auftritt, die ihr Maximum nach 17–24 Stunden erreicht und nach 3 Tagen sistiert; er führte diese Erscheinung auf positive Chemotaxis zurück. *Albu* hat im Blut des Kaninchens, nach Kochsalzinfusion zahlreiche Stechapfel- resp. Maulbeerformen der Erythrozyten beobachtet. Die gleiche Form hat E. *Albrecht* durch Zusatz von grosser Menge isotonischer Kochsalzlösung zum Blut in vitro gesehen und glaubte diese Erscheinung als Ionenwirkung des Kochsalzes ansprechen zu können. *Stern* hat nach Salzinfusion an Kaninchen das Hämoglobin in der Galle erwiesen.

Die Organalteration des Menschen durch Kochsalzinfusion wurde zuerst von *Rössle* mitgeteilt. Er hat bei der Autopsie von Leichen, welche kurz vor dem Tode Kochsalzlösung infundiert erhielten, als fast regelmässig charakteristischen Befund die trübe gelblich-graue Farbe des Herzmuskels bemerkt. Daneben hat er ebenso gelblich-graue Trübung von feuchter schwappender Beschaffenheit des Nierenparenchyms beobachtet.

Nürnberger hat unabhängig von Rössle ähnliche Befunde an Menschenmaterial beschrieben. Er hat weiter 1921, während meine Experimente schon im Gange waren, die Veränderungen des Blutbildes bei intravenöser Infusion verschiedener Lösungen beschrieben, indem er bei der Infusion von 500 ccm 0.9%ige Kochsalzlösung an gesunden Menschen mit vorangehender eine Stunde dauernder Leukopenie, Zunahme der neutrophilen Leukozyten, deutliche Hydrämie und leichte Abnahme der Lymphozyten beobachtete. Der Kurvengipfel der Leukozytose wird drei Stunden nach der Injektion erreicht, dann stieg die Kurve ziemlich rasch ab.

Über das Auftreten von Leukozytose durch Kochsalzzufuhr wurde weiter berichtet: durch subkutane Injektion von physiologischer Kochsalzlösung (*J. Rosenstern*), durch subkutane oder rektale Zufuhr von genügend grosser Dose Kochsalz beim Säugling (*S. Cobliner*). Die Ursache des Kochsalzfiebers ist noch nicht festgestellt.

E. Auf therapeutischer Seite ist die lebensrettende Wirkung der Kochsalzinfusion bei der Verblutung, bei grossen Wasserverlusten, wie bei Cholera und Brechdurchfall, und bei gewissen Vergiftungen bekannt.

Die konzentrierte Kochsalzlösung wirkt blutstillend. Ich habe die Untersuchung über die biologische Wirkung des Chlornatriums auf das Blut und die blutbildenden Organe durchgemacht. Bevor ich über die Resultate meines Versuches berichte, möchte ich meine Methodik kurz besprechen.

Methodisches: Um die antagonistische Wirkung anderer Salze auszuschliessen, benutzte ich das chemisch rein dargestellte Chlornatrium, welches durch Neutralisation von reinem Natrium bicarbonicum mit reiner Salzsäure dargestellt wurde. Das Experiment wurde an nüchternen Kaninchen durchgeführt. Die Kochsalzlös-

ung wurde ganz sorgfältig langsam in die Ohrvenen unter Kautelen der Aseptik eingeführt. Die Blutzählung wurde mit der Mischpipette von Thoma und der Zählkammer von Max-Levy ausgeführt. Den Hämoglobingehalt habe ich mit Hämometer von Sahli bestimmt und die abgelesene Skala angegeben. Alle Ausstrichpräparate wurden in absolutem Methylalkohol fixiert und mit Giemsa-Farblösung Grübler's gefärbt, und die Zählung der Leukozytenarten durchgeführt. Die Ergebnisse meines Experiments finden sich nachstehend.

I. 0.85%ige Kochsalzlösung, hypo- und hypertonische Kochsalzlösung.

Durch intravenöse Injektion von 0.85%iger Kochsalzlösung 2.0 ccm pro Kilo an 15 Kaninchen trat ausnahmslos auffallende Vermehrung der Leukozyten auf. An 7 Fällen davon wurde auch leichte temporäre Leukopenie 3–5 Minuten nach der Injektion beobachtet. Die gesamte Leukozytenzahl zeigte doppelte bis dreifache Vermehrung. Die Leukozytose begann meist eine Stunde nach der Injektion und dauerte 5–7 Stunden oder noch länger. Was die Leukozytenarten betrifft, so zeigten die pseudoeosinophilen Leukozyten die grösste prozentuale sowie absolute Zahl. Die Mastzellen und Monozyten zeigten auch leichte Vermehrung. Eine geringere Vermehrung der stabkernigen Leukozyten wurde beobachtet, Merkwürdigerweise nahm die prozentuale Zahl der Lymphozyten bedeutend ab, aber die absolute Zahl zeigte keine deutliche Schwankung. (Tab. I—V). Die Blutuntersuchung beider Ohrenvenen zeigte keine Unterschiede der Leukozytenzahl zwischen dem Blute der injizierten Venen und dem Blute des anderen Ohres. (Tab. VI u. VII). Die Zahl der roten Blutkörperchen und des Hämoglobingehalts zeigten keine deutliche Veränderung.

Tab. I. 0.85% NaCl-Lösung.

(Oben absolute zahl unten Prozentsatz)

Kaninchen Nr. 24. weiss ♀ 1.46 Kg. 14. Sept. 1921.									
Zeit nach der Inj.	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	6.46 2 ccm Prokilo Inj.	84	11080	44 0.4	177 1.6	221 2.0	0 0	5750 51.8	4900 44.2
5m			10600	42 0.4	85 0.8	212 2.0	0 0	5510 52.0	4760 44.8
30m			18800						
1h			22700	91 0.4	182 0.8	393 1.6	91 0.4	16600 73.2	5350 23.6
2h			28040	112 0.4	224 0.8	450 1.5	112 0.4	21220 75.6	5920 21.2
3h			26680						

4h	6.24		30120	120 0.4	240 0.8	602 2.0	240 0.8	22760 75.6	6140 20.4
5h			25680	103 0.4	206 0.8	514 2.0	206 0.8	18700 72.8	5960 23.2
7h	6.21	81	20920	83 0.4	167 0.8	418 2.0	83 0.4	15380 73.6	4770 22.8
27h	6.24	79	12880	51 0.4	154 1.2	206 1.6	0 0	7520 58.4	4950 38.4

Tab. II. 0.85% NaCl-Lösung.

Kanichen Nr. 25. weiss 2.135 Kg. ♀ 15. Sept. 1921.									
	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly
Vor der Inj.	5.62 2 ccm Prokilo Inj.	76	10200	41 0.4	163 1.6	163 1.0	0 0	5760 56.4	4080 40.0
5m	5.55		9870	39 0.4	79 0.8	158 1.6	0 0	5610 5.68	3990 40.4
1h			14240	57 0.4	114 0.8	228 1.6	0 0	9700 68.0	4160 29.2
2h			17160	69 0.4	137 0.8	343 2.0	0 0	12200 71.2	4380 25.6
3h			22360	89 0.4	358 1.6	447 2.0	89 0.4	16900 75.6	4470 20.0
4h			24040	96 0.4	192 0.8	482 2.0	96 0.4	18650 77.6	4530 18.8
5h			21640						
6h	5.41	73	22680	91 0.4	364 1.6	364 1.6	0 0	17700 78.0	4180 18.4
23h	5.64	75	10560	42 0.4	127 1.2	211 2.0	0 0	6210 58.8	3970 37.6

Tab. III. 0.85% NaCl-Lösung.

Kaninchen Nr. 26. (Schwarz weiss fleckig) ♀ 3.61 Kg. 18. Sept. 1921.									
Zeit	R.	Hb.	W.	E.	B.	M.	St.	Ps.	Ly.
Vor der Inj.	6.32 2 ccm Prokilo Inj.	84	8240	33 0.4	66 0.8	132 1.6	0 0	3860 46.8	4150 60.4
5m	6.30		7760						
1h			11400						
2h			17600	70 0.4	282 1.6	352 2.0	0 0	12520 71.2	4360 24.8

3h			25720						
4h			38360	153 0.4	614 1.6	614 1.6	0 0	32500 83.6	4910 12.8
5h			25400						
7h	6.20	81	18720	75 0.4	300 1.6	374 2.0	0 0	13620 72.8	4340 23.2
23h	6.38	84	8760	70 0.4	175 2.0	175 2.0	0 0	4340 49.6	3990 45.6

Tab. IV. 0.85% NaCl-Lösung.

Kaninchen Nr. 31. weiss ♀ 2.745 Kg. 5. Opt. 1921.									
	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	6.35 2 ccm prokilo Inj.	82	9280	74 0.8	148 1.6	185 2.0	0 0	4120 44.4	4750 51.2
5m	6.01		8680						
1h			10880						
2h			15000						
3h			22200	89 0.4	355 1.6	355 1.6	0 0	16140 72.8	5240 23.6
4h			29600	118 0.4	356 1.2	473 1.6	0 0	23080 78.0	5560 18.8
5h			37440	150 0.4	450 1.2	600 1.6	0 0	30710 82.0	5550 14.8
6h			41640	166 0.4	500 1.2	832 2.0	0 0	34270 82.4	5820 14.0
7h			22520						
8h			17120	68 0.4	274 1.6	342 2.0	0 0	11160 65.2	5280 30.8
24h	6.38	77	12440	50 0.4	99 0.8	249 2.0	0 0	6920 55.6	5130 41.2

Tab. V. 0.85% NaCl-Lösung.

Kaninchen Nr. 32. weiss ♀ 1980 Kg. 8. Okt. 1921.									
	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	6.15 2 ccm Prokilo Inj.	81	9480	38 0.4	153 1.6	228 2.4	0 0	3140 33.2	5920 62.4
5m	6.07		8840						

30m			13640						
1½h			14840	59 0.4	119 0.8	297 2.0	0 0	8260 55.6	6120 41.2
2½h			16880						
3½h			22400	90 0.4	179 0.8	358 1.6	90 0.4	15150 67.6	6550 29.2
4½h			21200						
5½h			15700						
6½h	5.92	77	12840	51 0.4	103 0.8	257 2.0	0 0	6740 42.4	5710 44.4

Tab. VI. 0.85% NaCl-Lösung.

Kaninchen Nr. 60. weiss ♀ 1.940 Kg. 21. Dec. 1921.

Zeit nach der Inj.	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	7.10 2 ccm Prokilo Inj.	90	8440	33 0.4	33 0.4	168 2.0	0 0	4270 50.8	3900 46.4
3m			7120	28 0.4	28 0.4	57 0.8	0 0	4010 56.4	2990 42.0
30m			14400	57 0.4	115 0.8	230 2.6	0 0	9900 68.8	4080 28.4
1h	68.1		14960	59 0.4	119 0.8	180 1.2	0 0	10900 73.6	3540 24.0
2h	(nicht injicierte Seite)		18260	73 0.4	73 0.4	367 2.0	0 0	14100 77.2	3670 20.0
	(injicierte Seite)		17900						
3h	6.61		17600	0	140 0.8	255 1.2	0 0	13500 76.8	3730 21.2
4h			20160	0 0	80 0.4	322 1.6	0 0	15500 77.2	4180 20.8
5h	6.89	81	22400	0 0	179 0.8	448 2.0	0 0	17900 80.0	3850 17.2
6h			32200	0 0	238 0.4	276 0.8	138 0.4	27600 86.0	3900 12.4
23h	6.67	78	9120	36 0.4	86 0.4	73 0.8	73 0.8	4760 52.4	4110 45.2

Tab. VII. 0.85% NaCl-Lösung.

Kaninchen Nr. 61. Weiss (Schwarzfleckig) ♀ 2.370 Kg. 22. Dec. 1921.									
Zeit nach der Injekt	R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	5.88 2 ccm Prokilo Inj.	82	9040	0 0	36 0.4	108 1.2	0 0	4160 46.4	4680 52.0
3m			8800	0 0	0 0	105 1.2	0 0	3450 39.2	5240 59.6
30m			12720	0 0	100 0.8	203 1.6	50 0.4	6750 53.2	5590 44.0
1½h	nicht injicierte Seite)		15800	0 0	189 1.2	316 2.0	63 0.4	9539 60.4	5690 36.0
	5.74 injicierte Seite)		16560						
2½h			24920	0 0	99 0.4	498 2.0	298 1.2	17700 71.2	6280 25.2
3½h	6.03		26400	0 0	210 0.8	420 1.6	210 0.8	17720 67.2	7800 29.6
4½h			22000	0 0	88 0.4	352 1.6	176 0.8	15300 69.6	5630 25.6
5½h	5.78	79	23160	0 0	184 0.8	462 2.0	92 0.4	15730 68.4	6550 28.4
6½h			18680	0 0	149 0.8	298 1.6	74 0.4	12950 69.6	5130 27.6
23h	6.20	86	10880	0 0	86 0.8	86 0.8	0 0	5780 53.6	4840 44.8

Bei der Anwendung von Aqua destillata sowie 0.3% Kochsalzlösung zeigte sich keine deutliche Schwankung der Leukozytenzahl. Die Injektion von 0.6%iger Kochsalzlösung rief doppelte Vermehrung des Leukozytenwerts hervor und dauerte 4 Stunden lang.

Durch die Injektion von 0.9%, 1.7% oder 3.4% Kochsalzlösung habe ich die gleichen Resultate wie bei der 0.85%igen beobachtet, Injektion von 10.0%, 25.0% oder 26.4% (gesättigte Kochsalzlösung bei Zimmertemperatur) Lösung zeigten doppelte Vermehrung der Leukozytenzahl, dabei verminderten sich die roten Blutkörperchen sowie der Hämoglobingehalt in geringerem Grade.

Die Verteilung der Leukozyten zwischen Ohrvenen und Eingeweiden bei der Injektion von 0.85%iger Kochsalzlösung wurden ebenfalls untersucht. Wie aus Tabelle VIII und IX ersichtlich, gingen die Zahlen der weissen sowie der roten Blutkörperchen an beiden Stellen Hand in Hand, wobei hauptsächlich die Leukozytenzahl schwankte.

Tab. VIII. 0.85% NaCl-Lösung.

Kaninchen Nr. 53. weiss ♀ 2.080 Kg. 14. Dec. 1921.										
Zeit nach der Inj.		R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	Ohr	7.13		9930	119 1.2	79 0.8	119 1.2	0 0	4500 45.2	5140 51.6
	Leber	7.04 2 ccm Prokilo Inj.		8820	100 1.2	33 0.4	66 0.8	0 0	3990 48.0	4130 49.6
1h	Ohr			12640						
	Leber			11400						
2h	Ohr			14480	116 0.8	58 0.4	290 2.0	0 0	8750 60.4	5270 36.4
	Leber			12360	49 0.4	148 1.2	197 1.6	49 0.4	7560 61.2	4350 35.2
3h	Ohr			17440						
	Leber			14320						
4h	Ohr			12320	99 0.8	148 1.2	247 2.0	0 0	7010 56.8	4840 39.2
	Leber			10560	42 0.4	84 0.8	215 2.0	0 0	5880 56.8	4220 40.0
(durch Luftembolie getötet)										

Tab. IX. 0.85% NaCl-Lösung.

Kaninchen Nr. 62. weiss ♀ 1.95 Kg. 7. Jan. 1922.										
Zeit nach der Inj.		R.	Hb.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	Ohr.	5.94	87	11320	90 0.8	181 1.6	270 2.4	0 0	5861 52.0	4870 43.2
	Leber	5.96 2 ccm Prokilo Inj.		9280	37 0.4	110 1.2	110 1.2	0 0	4930 53.2	4070 44.0
30m	Ohr.			17280	69 0.4	236 1.2	276 1.6	0 0	12100 70.8	4500 26.0
	Leber			—	—	—	—	—	—	—
1h	Ohr			19800	79 0.4	396 2.0	396 2.0	157 0.8	13500 68.0	5300 26.8
	Leber			11520	46 0.4	138 1.2	184 1.6	92 0.8	7400 64.4	3640 31.6
2h	Ohr			19520	0 0	390 2.0	468 2.4	78 0.4	14200 73.2	4300 22.0

2h	Leber			17160	68 0.4	220 1.2	340 1.8	68 0.4	12800 75.0	3630 21.2
3h	Ohr			27720	0 0	442 1.6	665 2.4	220 0.8	20800 75.6	5430 19.6
	Leber			16760	0 0	200 1.2	133 0.8	133 0.8	12900 77.4	3310 19.8
4h	Ohr			21920	0 0	350 1.6	350 1.6	175 0.8	16400 74.0	4800 22.0
	Leber			16520	0 0	198 1.2	264 1.6	66 0.4	12550 76.6	3300 20.2
5h	Ohr			26240	0 0	420 1.1	735 2.8	104 0.4	20600 78.4	4440 16.8
	Leber			14960	0 0	60 0.4	180 1.2	120 0.8	11480 76.6	3150 21.0
(durch Luftembolie getötet)										

Zum Kontrollversuch benutzte ich weiter die isomolekulare Lösung mit 0.85%iger Kochsalzlösung, nämlich 1.49% Bromnatriumlösung und 2.34% Natriumsulfatlösung; dabei fand die gleiche Leukozytose wie bei 0.85% Kochsalzlösung statt. Die absolute und relative Zahl der pseudoeosinophilen Leukozyten vermehren sich am meisten, während die Lymphozyten fast den gleichen absoluten Wert wie vor der Injektion behielten.

Die Injektion von 0.85% Kochsalzlösung, 2 ccm pro Kilo, welche durch den Zusatz von Natrium bicarbonicum im Verhältnis von 0.01% schwach alkalisiert wurde, rief auch dreifache Vermehrung der Leukozyten hervor and dauerte 5–7 Stunden lang.

II. Antagonistische Salzwirkung.

Durch die Injektion 2.0 ccm pro Kilo von folgenden Salzmischungen trat doppelte bis dreifache Vermehrung der Leucozyten auf, und so wirkten solche beigefügten Salze nicht als Antagonisten in diesen Verhältnissen:

NaCl 0.85%	NaCl 0.85%
$CaCl_2$ 0.02%	$CaCl_2$ 0.06%
	K Cl 0.06%
NaCl 0.85%	NaCl 0.83%
$CaCl_2$ 0.02%	$CaCl_2$ 0.1%
K Cl 0.02%	K Cl 0.1%

Nach den Versuchen mit verschiedenen gemischten Salzlösungen gelang es mir endlich, durch die Anwendung von Salzgemisch

NaCl 0.85% die antagonistische Wirkung zu sehen, indem ich mit

$BaCl_2$ 0.04%

diesem Salzgemisch keine Leukozytose erzeugen konnte. Das Blutbild zeigte trotz der Injektion dieses Salzgemisches nur physiologische Schwankung. Beim späteren Versuch mit 0.85%iger Kochsalzlösung an dem Kaninchen, welches mit dem obengenannten Salzgemisch vorbehandelt wurde, zeigte sich auch hochgradige Leukozytose. 0.04% Baryumchlorid allein verursachte starke Leukozytose. (Tab. X — XII).

Tab. X.

(I) Kaninchen Nr. 51. 2.15 Kg. weiss ♀							
1. Dec. 1921.				5. Dec. 1921.			
	R.	Hb.	W.		R.	Hb.	W.
Vor der Inj.	7.36	86	10240	Vor der Inj.	7.37	87	10920
NaCl 0.85% / $BaCl_2$ 0.04% } 2 ccm Prokilo Inj.				NaCl 0.85 2 ccm Prokilo Inj.			
30m			12640	30m			12280
1h			13040	1h			13600
2h	7.07		11800	$1^1/_2$h			19240
3h			12080	$2^1/_2$h			22600
4h			12600	$3^1/_2$h			18640
5h	7.21		14680	$4^1/_2$h			16760
6h			12320	$5^1/_2$h			15920
7h	7.09	80	10520	$6^1/_2$h	7.20	82	10400

Tab. XI.

(II) Kaninchen Nr. 57. 2.07 Kg. weiss (schwarz fleckig) ♀							
30. Nov. 1921.				3. Dec. 1921.			
Vor der Inj.	7.15	92	8640	Vor der Inj.	7.19	90	8920
NaCl 0.85% / $BaCl_2$ 0.04% } 2 ccm Prokilo Inj.				NaCl 0.85% 2 ccm Prokilo Inj.			
30m			10160	30m			8480
1h			9760	1h			10920
2h	7.13		11040	2h	7.22		15000
3h			12720	3h			16800
4h			10560	4h	7.25		18400
5h	7.26	86	13800	5h			15840
6h			9760	6h	7.12	88	8720

Tab. XII.

Kaninchen Nr. 145. (weiss schwarz fleckig) ♀ 2.14 Kg. 19. Jan. 1922.				Kaninchen Nr. 146. weiss ♀ 2.29 Kg. 20. Jan. 1922.			
	R.	Hb.	W.		R.	Hb.	W.
Vor der Inj.	5.86	77	8840	Vor der Inj.	6.50	86	9800
$BaCl_2$ 0.04% 2 ccm Prokilo Inj.				$BaCl_2$ 0.04% 2 ccm Prokilo Inj.			
30m	5.77		11400	30m	6.39		11880
1h			10720	1h			11040
2h			14840	2h			17480
3h			18700	3h	6.32		20880
4h			17320	4h			23880
5h	5.89	72	12320	5h	6.38	83	16920
6h			10800	6h			17760

III. 10.0%ige Kochsalzlösung.

Die Blutplättchenzählung bei der Injektion von 10%iger Kochsalzlösung wurde ausgeführt. Dabei wurden neben den Blutplättchen auch die Blutkörperchen gezählt. Die durchschnittliche Zahl der Blutplättchen der 22 gesunden Kaninchen beträgt 654,800 in 1 mm^3 (606,000–726,000). Die Form der Blutplättchen ist meist rundlich oder oval, selten wurstförmig. Der Durchmesser der mittelgrossen Blutplättchen misst 3.0–9.8 μ der kleineren 1.8–2.3 μ, der grösseren 5.4 μ. Die Grösse der wurstförmigen Riesenplättchen erreicht 2.5–3.9 μ Breite, 7.7–8.5 μ Länge. Die Grundsubstanz der Blutplättchen, nach der Methode *Fonzi*'s fixiert, färbt sich mit Giemsa's Farblösung ganz homogen blass rötlich violett; der Rand ist mit sehr dünner Linie von rötlich violetter Farbe scharf begrenzt; in der Grundsubstanz befinden sich gröbere violette Granula zerstreut oder gruppiert. Die Struktur der grösseren Blutplättchen und der Riesenplättchen sieht anders aus; in der blass rötlich violetten Grundsubstanz verteilen sich ganz diffus zahlreiche feinere violette Granula. Diesen Blutplättchen fehlt die scharfe Randlinie. Aus meinen 17 Versuchen der Injektion von 10.0% Kochsalzlösung 1 ccm pro Kilo gehen die folgende Resultate hervor. In jedem Falle dieser Injektion sieht man 60–76% Vermehrung der Blutplättchen schon 30 Minuten nach der Injektion. Binnen 6 Stunden erhielt die Blutplättchenzahl doppelten bis dreifachen Wert des normalen. Die Zunahme betrifft wesentlich mittelgrosse Blutplättchen. Die Leukozyten sind durchschnittlich doppelt vermehrt, die roten Blutkörperchen und der Hämoglobingehalt sind immer in geringem Grad vermindert. Durch die Resistenzbestimmung der Erythrozyten gegen hypotonische Kochsalzlösung nach

v. Limbeck's Methode ergab sich, dass die Erythrozyten 10 min. 30 min. 1 Stunde, 2 Std. 4 Std. 5 Std. und 6 Std nach der 10%igen Kochsalzinjektion keine deutliche Veränderung der Resistenz zeigten (Tab. XX). Durch dieses Experiment ist die Vermehrung der Blutplättchen nach der Injektion von 10% Kochsalzlösung festgestellt (Tab. XIII — XIX). Die Injektion von 25.0% NaCl-Lösung sowie von 27.6% Natriumsulfatlösung (isomolekulare Lösung für 10% NaCl-Lösung in Bezug auf Na-Molekül) rief gleiche Blutplättchenvermehrung wie bei der 10%igen Kochsalzlösung hervor. Durch die Injektion von 50% Traubenzuckerlösung (annähernd isotonisch für 10% NaCl-Lösung) habe ich 55–70% Zunahme der Blutplättchen gesehen.

Tab. XIII. 10.0% NaCl-Lösung.

Kaninchen Nr. 78. weiss ♀ 2.66 Kg. 9. März. 1922.											
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.	Pl.
Vor der Inj.	6.09 1 ccm Prokilo Inj.	78	608,000	9120	73 0.8	109 1.2	182 2.0	18 0.2	3900 42.8	4820 52.8	18 0.2
1h	6.08		1,050,000	10960	88 0.8	131 1.2	170 1.6	0 0	5380 49.2	5150 47.0	22 0.2
2h				21480	344 1.6	172 0.8	344 1.6	86 0.4	14700 68.4	5850 27.2	
3h	6.13		1,077,000	18920	151 0.8	151 0.8	303 1.6	76 0.4	12860 68.0	5380 28.4	
4h				15240							
5h				15680							
6h	59.4	74	1,370,000	9960	79 0.8	79 0.8	159 1.6	0 0	5100 51.2	4540 45.6	

Tab. XIV. 10% NaCl-Lösung.

Kaninchen Nr. 82. weiss ♀ 2.30 Kg. 28. März. 1922.										
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.
Vor der Inj.	6.54 1 ccm Prokilo Inj.	82	641,000	8040	0 0	64 0.8	96 1.2	0 0	3630 45.2	4240 52.8
1h	6.60		894,000	8260	0 0	67 0.8	67 0.8	0 0	3950 47.2	4280 51.2
2h				11200	0 0	45 0.4	89 0.8	0 0	5730 51.2	5340 47.6
3h	6.62		1,183,000	11640	0 0	93 0.8	140 1.2	0 0	6150 52.8	5260 45.2
4h				17080	0 0	136 0.8	342 2.0	0 0	10380 60.8	6220 36.4

5h				9920	0 0	79 0.8	79 0.8	0 0	4760 48.0	5000 50.4
6h	6.42	79	1,424,000	8960	0 0	36 0.4	143 1.6	0 0	4550 50.8	4230 47.2

Tab. XV. 10% NaCl-Lösung.

Kaninchen Nr. 88. weiss ♂ 2.20 Kg. 25. April. 1922.											
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.	Pl.
Vor der Inj.	6.83 1 ccm Prokilo Inj.	87	679,000	7800	62 0.8	62 0.8	123 1.6	0 0	3470 45.6	3890 51.2	
2h	6.68		1,230,000	12560	101 0.8	50 0.4	101 0.8	0 0	6890 64.6	5420 4.32	
3h				16880	135 0.8	67 0.4	270 1.6	0 0	10050 59.6	6350 37.6	
4h	6.65		1,488,000	18000	144 0.8	27 0.4	288 1.6	0 0	11720 65.2	5760 32.0	36 0.2
6h	6.70	83	1,580,000	10440	42 0.4	42 0.4	167 1.6	0 0	5600 53.6	4600 44.0	
26 April	6.86 1 ccm Prokils Inj. (2.15 Kg.)	87	1,008,000	8420	34 0.4	34 0.4	67 0.8	0 0	3770 44.8	4510 53.6	
2h	6.70		1,630,000	11960							
4h				18680	95 0.8	84 0.4	95 0.8	0 0	7600 63.6	4120 34.4	
5½h	6.54	84	1,790,000	10480	84 0.8	84 0.8	167 1.6	0 0	6040 57.6	4110 39.2	

Tab. XVI. 10.0% NaCl-Lösung.

Kaninchen Nr. 196. weiss ♀ 2.22 Kg. 22. Jun. 1922.										
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.
8h A.M. 22 Jun.	6.04	12	626,000	8490	67 0.8	67 0.8	136 1.6	0 0	3800 44.8	4400 52.0
9h A.M. 27 Jun.	6.08 1 ccm Prokilo Inj. (2230 g.)	84	643,000	8760						
30m	6.07		1,002,000	16460	131 0.8	131 0.8	262 1.6	0 0	8400 51.2	7480 45.6
5h	6.05	83	1,223,000	11800	94 0.8	94 0·8	189 1.6	0 0	6900 58.4	4540 38.4

Tab. XVII. 10.0% NaCl-Lösung.

Kaninchen Nr. 200. weiss ♀ 2.03 Kg. 6. Juli. 1922.										
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.
8h A. M. 6 Juli	6.11	83	617,000	7820	26 0.8	31 0.4	31 0.4	0 0	3500 44.8	4100 53.6
10h A.M. 60 Juli	6.13 1 ccm Prokilo Inj.		622,000	8080	64 0.8	16 0.2	32 0.4	0 0	3820 47.4	4140 51.2
30m	6.10		1,067,000	13280						
3h	6.08		1,735,000	14440	58 0.4	58 0.4	115 0.8	0 0	9360 64.8	4850 33.6
6h	6.07	82	1,690,000	10480	84 0.8	4.2 0.4	84 0.4	0 0	6800 64.8	3480 33.2
7 Juli	6.18 1 ccm Prokilo Inj. (2020 g.)	85	970,000	10960	87 0.8	175 1.6	87 0.8	0 0	6920 63.2	3680 33.6
3h	6.13		1,540,000	15320	61 0.4	124 0.8	61 0.4	0 0	9560 62.4	5500 36.0
7h	6.08	82	1,770,000	9560	76 0.8	38 0.4	38 0.4	0 0	5500 57.6	3900 40.8
(durch Luftembolie getötet)										

Tab. XVIII. 10% NaCl-Lösung.

Kaninchen Nr. 201. weiss ♀ 1.96 Kg. 10. Juli 1922.										
Zeit nach d. Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.
8h A. M. 10 Juli	6.28	85	715,000	10520	42 0.4	84 0.4	42 0.4	0 0	5050 48.0	5300 50.4
10h A. M. 10 Juli	6.23 1 ccm Prokilo Inj.		696,000	10550	84 0.8	42 0.4	42 0.4	0 0	5380 51.2	4960 47.2
30m	6.18		1,260,000	20280	164 0.8	164 0.8	82 0.4	0 0	12900 63.0	7200 35.0
4h	6.24		1,784,000	15360	61 0.4	123 0.8	61 0.4	0 0	10200 66.4	4910 32.0
11 Juli	6.17 1 ccm Prokilo Inj. (1970 g.)	82	870,000	10280	82 0.8	123 1.2	82 0.8	0 0	6010 58.4	3980 38.8
2h	6.12		1,940,000	13520	0 0	180 0.8	180 0.8	27 0.2	9200 68.0	4110 30.2
6h	6.15	82	1,910,000	13920	55 0.4	111 0.8	55 0.4	0 0	9580 68.8	4120 29.6
(durch Luftembolie getötet)										

Tab. XIX. 10.0% NaCl-Lösung.

Kaninchen Nr. 202. Schwarz (weiss fleckig) ♂ 1.98 Kg. 12. Juli 1922.										
Zeit nach der Inj.	R.	Hb.	Pt.	W.	E.	B.	Mo.	St.	Ps.	Ly.
8h A. M. 12. Juli	6.20	82	606,000	8200	131 1.6	33 0.4	65 08	0 0	4010 48.8	3900 47.6
10h A. M. 12. Juli	6.31 1 ccm Prokilo Inj.		611,000	8440						
30m	6.29		1,071,000	13000	104 0.8	104 0.8	208 1.6	0 0	8220 63.2	4370 33.6
4h	6.21	80	1,404,000	10720	171 7.6	86 0.8	86 0.8	0 0	6600 61.6	3780 35.2

Tab. XX.

Resistenzbestimmung der Erythrozyten nach Kochsalzinjektion. (10%) 20. Dez. 1922.												
Kaninchen	Körp. gew.	Vor der Injektion		Zeit nach der Injektion 2 ccm Prokilo.								
				10m		30m		1h		2h		
		min Resist	rote Nuance	min Resist	rote Nuance	min Resist	rote Nuance	min Resist	rote Nuance	min Resist	rote Nuance
Nr. 242	2.95 Kg. ♀	0.52	0.50			0.54	0.52	0.52	0.50	0.54	0.52
Nr. 243	2.50 Kg. ♀	0.52	0.50			0.54	0.52	0.52	0.50	0.52	0.50
Nr. 244	3.15 Kg. ♂	0.52	0.50	0.54	0.52			0.52	0.50	0.54	0.52
Nr. 245	2.60 Kg. ♀	0.52	0.50	0.54	0.52			0.54	0.52	0.54	0.52

Kaninchen	Körp. gew.	Vor der Injektion		Zeit nach der Injektion 1 ccm Prokilo.					
				10. Juli 1922		4h		5h		6h	
		min Resist	rote Nuance			min Resist	rote Nuance	min Resist	rote Nuance	min Resist	rote Nuance
Nr. 201	1.96 Kg. ♀	0.50	0.48					0.50	0.48	0.50	0.48
Nr. 216	2.41 Kg. ♀	0.52	0.50					0.52	0.50	0.52	0.50
Nr. 217	2.30 Kg. ♂	0.50	0.48					0.50	0.48	0.52	0.50
				12. Juli 1922							
Nr. 202	1.98 Kg. ♂	0.52	0.50			0.52	0.50				
Nr. 212	2.12 Kg. ♀	0.50	0.48			0.50	0.48				
Nr. 214	2.25 Kg. ♀	0.50	0.48			0.52	0.50				

IV. Agglutinine und bakterizide Kraft.

Ich möchte hier noch eine kurze Erörterung der Versuche vornehmen, wo der Immunkörper durch die Kochsalzinfusion beeinflusst wurde. 7 Kaninchen, welche schon anderthalb bis drei Monate vorher mit Cholera- resp. Typhuskultur vorbehandelt waren, wurde 0.85%ige Kochsalzlösung, 5 ccm pro Kilo eingeführt. Die Vermehrung der Leukozytenzahl zeigte doppelt bis vierfachen Wert. Die bakterizide Kraft wurde nach Neisser und Wechsberg's Plattenverfahren bestimmt, und der höchste Verdünnungstiter, wo das Bakterienwachstum auslieb, wurde angegeben. Aus diesem Verfahren sowie aus der Agglutinationsprobe ergab sich, dass die Agglutinine und bakterizide Kraft nach der Leukozytose sich vermehrten, welche durch Kochsalzinfusion hervorgerufen war. (Tab. XXI. u. XXII.)

Tab. XXI.

Kaninchen Nr. 166 weiss ♀ 3.46 Kg. 7. März. 1922.					
Dose der eingespritzten Cholera-Kultur: 1, 2, 5, 7, mgr. Letzte Injektion 8. Dec. 1921.					
	R.	Hb.	W.	Titer der Agglut.	Titer der bakterizid Kraft.
Vor der Inj.	6.26	79	9920	1:2000	1:500
	10h A.M. NaCl 0.85% 5 ccm Prokilo Inj.				
1h			12100		
2h			17380		
3h			23880	1:2000	
4h			37480		
5h			24400		
9h 40m A.M. 8. März	4h P.M. NaCl 0.85% 5 ccm Prokilo Inj.				
	6.19	75	11660	1:10000	1:5000
	10h A.M. NaCl 0.85% 5 ccm Prokilo Inj.				
3h	5.96	77	29320		
7h	6.12	78	12740	1:10000	
4h P.M. 10. März	6.14		11600	1:2000	1:1000

Tab. XXII.

Kaninchen Nr. 167 (schwarz (weiss fleckig)) ♀ 2.85 Kg. 8. März 1922.					
Dose der eingespritzten Cholera-Kultur: 2, 5, 7, 8. mgr. Letzte Injektion 22. Dec. 1921.					
	R.	Hb.	W.	Titer der Agglut.	Titer d. bakteriziden Kraft.
Vor der Inj.	5.88	76	7960	1:1000	1:1000

	9h 30m A.M. NaCl 0.85% 5 ccm Prokilo Inj.				
30m			12320		
1h			12240		
2h			17560		
3h	6.10		27400		
4h			18960		
5h			13240		
7h	5.97	77	8560	1:5000	
9h 15m A.M. 9. März	5h P.M. NaCl 0.85% 5 ccm Prokilo Inj.				
	5.83	77	9720		
	9h 30m A.M. NaCl 0.85% 5 ccm Proklio Inj.				
3h			26120		
7h	6.08	79	12040	1:10000	1:5000
3h P.M. 11. März	6.14	81	10760	1:2000	

V. Pathologisch-anatomische Veränderungen.

Weiter möchte ich die pathologisch-anatomischen Befunde der Versuchskaninchen erwähnen, welche mit 0.85%iger Kochsalzlösung, 2 oder 5 ccm pro Kilo ein bis dreimal, resp. 10.0% NaCl-Lösung, 1 ccm pro Kilo ein bis 5mal mit Intervall von einem halben oder zwei Tagen, injiziert wurden. Das Körpergewicht und der allgemeine Zustand blieben dadurch fast unverändert. Die auffallendsten Befunde sind in den hämatopoetischen Organen zu sehen. In dem Knochenmarkt wird eine Vermehrung der pseudoeosinophilen Zellen bemerkt, welche bei der Injektion von 0.85% NaCl-Lösung in die Blutgefässe wandern und das Phänomen der Leukozytose verursachen (Fig. 3). Nach wiederholten Injektionen von 10% NaCl-Lösung tritt die Vermehrung der Megakaryozyten auf, zugleich wird die vermehrte Embolie der nackten Kerne von Megakaryozyten in den Kapillaren der Alveolensepten der Lunge beobachtet. Das Knochenmark war allgemein blutreich und ödematös.

Die Lymphknoten zeichnen sich durch eine Wucherung der Retikuloendothelien mit einer hochgradigen Erythrophagie derselben aus. Dies habe ich hauptsächlich in den Hals-, Inguinal- und Retrosternaldrüsen gefunden, in der Mesenterialdrüse nur im geringen Grade, dagegen niemals in dem lymphatischen Gewebe der Darmwand und des Wurmfortsatzes. Die Marksubstanz der Lymphdrüse sah makroskopisch dunkelrot aus. Mikroskopisch waren in hochgradigen Fällen die Erythrozyten von fast allen Reticuloendothelialzellen der Marksubstanz

und der Randsinus massenhaft phagozytiert, und aufs dichteste nebeneinander in diesen Zellleibern liegend. Die Kerne der Reticuloendothelien lagen bald im Zentrum des Zellleibes, bald an den Rand gedrückt. Die gewucherten Reticuloendothelien sind in der Mehrzahl als freie Rundzellen (Histiozyten) im Lumen des Marksinus vorhanden. Die phagozytierten Erythrozyten waren rundlich, meist wie gewöhnlich gefärbt, aber einige davon nur schwach. Der Lymphsinus war im allgemeinen erweitert und ödematös, die Lymphfollikel meistens verkleinert. Die Keimzentren meist nicht deutlich. Im frühen Stadium der Erythrophagie wurden Ansammlungen von zahlreichen Erythrozyten betroffen, die noch die eigentlichen Napfform der Erythrozyten bewahrten, und die sich um die Reticuloendothelien herum gruppierten. Einige Anzahl von solchen Erythrozyten drangen schon zum Teil rosettenartig anordnend in den Zellleib der Reticuloendothelien hinein, und bilden oft eine schöne Figur von Chrysanthemumblumen (Fig. 1 und 2).

Bei jeder Sektion der Versuchstiere wurde eine besondere Aufmerksamkeit darauf gelenkt, ob in den regionären Gewebe eine Blutung stattfindet oder nicht. Ich konnte aber niemals eine Blutung finden. Die Herkunft der phagozytierten Erythrozyten sollen demnach die jenige des kreisendes Blutes sein. Auch das Fehlen oder Minimale Anwesenheit der Erythrozyten im Randsinus der Lymphknoten im Gegensatz zum Marksinus sprechen nicht für einer vorher stattgefundenen Blutung in irgend einer Stelle des Körpers. Im weiteren Verlauf dieser Veränderung trat Ansiedelung von Hämosiderinkörnchen in den Lymphknoten auf, oft wurde allerdings nur Spur solcher Hämosiderinansiedelung beobachtet.

Die Milz zeigte im allgemeinen Zunahme an Grösse und Gewicht und war reich an Blut. Die Milzknötchen waren meist verkleinert. Nach wiederholten Injektionen von 10% NaCl wurde Vermehrung der gelb- bis dunkelbraunen Pigmentkörnchen in der Pulpa bemerkt, welche auf Eisenreaktion schön blau verfärbten.

Der Rindenteil des Thymus ist etwas verschmälert. Eine Vermehrung der eosinophilen Zellen wurde in dem Thymus beobachtet. Die Niere war leicht hyperämisch, übrigens ohne Besonderheiten. Die Kupfferschen Sternzellen enthielten reichliche Hämosiderinkörnchen, bei höherem Grad des Erythrozytenzerfalls auch in den Leberzellen wurden feine Hämosiderinkörnchen beobachtet.

Um weiter dieser Frage eingehenden Schluss zu ziehen habe ich folgenden Versuche angestellt, welche im Stande wären, welch eine der beiden, ob Erythrozyten selbst oder die Reticuloendothelien, beschuldigt werden müssen für die genannte lebhafte Erscheinung, die Erythrophagie.

VI. Transfusion und Autotransfusion.

Endlich möchte ich noch einiges über die Transfusion zufügen. Das Blut eines Kaninchens, welchem 6 Stunden vorher 10% NaCl-Lösung injiziert worden war, wurde einem zweiten Kaninchen in die Ohrvenen eingeführt. Einem anderen

Kaninchen wurde das Blut eines gesunden Kaninchens injiziert. Bei der Transfusion wurde möglichst intaktes Blut benutzt, indem ich dem Blut keine Substanz zufügte, nur mit abgekühlter Spritze die Gerinnung vermeidend. Als Kontrollversuch wurde die Autotransfusion ausgeführt, nämlich das von der Vene eines Ohres entnommene Blut wurde sofort in die Vene des anderseitigen Ohres eingeführt. Jedem Kaninchen wurden direkt vor der Transfusion 10 ccm Blut entnommen und darauf die gleiche Menge Blut eingeführt. 20 Stunden nach der Transfusion wurde in jedem Fall hochgradige Erythrophagie in den Lymphdrüsen beobachtet. In der Lymphdrüse und der Milz wurden viele atypische eosinophile Zellen angesammelt oder zerstreut in den Markssträngen der Lymphdrüsen sowie in dem peripheren Teil der Milzknötchen und der Lymphfollikel gefunden. Diese eosinophilen Zellen zeichnen sich durch stäbchenförmige oder spindelförmige Granula von matt roter Farbe aus. Beide Enden der Granula waren abgerundet oder scharf geschnitten. Darunter konnte man auch normale eosinophile Zellen finden. Solche atypische eosinophile Zellen konnte man wohl auch bei der Injektion von 10% NaCl-Lösnng in der Milz und Lymphdrüse beobachten. Aus den obenerwähnten Befunden könnte man vermuten, dass die atypischen eosiniphilen Zellen eine gewisse Beziehung zu dem Zerfall der Erythrozyten haben. Die Milz war voluminös und reich an Blut.

Epikrise und Zusammenfassung.

Betreffes der Entstehung der Leukozytose bei der Injektion von Kochsalzlösung kann man drei Möglichkeiten annehmen.

1. Chemotaktische Erscheinung. In früheren Zeiten wurde die Leukozytose auf chemotaktische Wirkung zurückgeführt. Aber neuere Untersuchunge legen das Schwergewicht bei des Entstehung der Leukozytose weniger auf chemotaktische Wirkung sondern mehr auf die biologische Tätigkeit u. z. auf die Reaktionsfähigkeit und Funktionstätigkeit der blutbildenden Organe, insbesondere des Knochenmarkes. Vor allem vertritt dies Naegeli mit seinem Wort „Der chemische Körper macht nicht direkt Leukozytenansammlung, sondern er beeinflusst die Funktion, die Leukopöesie des Knochenmarkes, und jetzt kann unter Umständen eine Vermehrung der Leukozyten eintreten."

Die Blutuntersuchung wurde stets an dem nicht injizierten Ohr vorgenommen. Als Kontrollversuch habe ich auch an beiden Ohren, an dem Zentralteil der injizierten Ohrvenen und an der Vene des anderseitigen Ohres die Blutuntersuchung ausgeführt. Dabei zeigte die Differenz der Leukozytenwerte an beiden Seiten nur 2.0 und 4.6% (Tab. VI u. VII). So kann man mindestens die lokale Ansammlung der Leukozyten ausschliessen.

2. Die ungleiche Verteilung der Leukozyten und die stärkere Ansammlung in der Peripherie bei der Leukozytose, wie *Kuder* und *Schulz* behaupteten, ist bei meinen Fällen durch die Ergebnisse der Blutuntersuchung an der Ohrvene sowie an der Leber auszuschliessen (Tab. VIII & IX).

3. Der Ursprung der Leukozytose aus dem Knochenmark wurde von vielen Autoren unzweifelhaft bewiesen. Die Leukozytose wird als der morphologische Ausdruck der funktionellen Änderung des Knochenmarks angesehen. Bei meinen Experimenten ist die Leukozytose wohl als Folge des Reizes am Knochenmark durch Kochsalzinjektion zu betrachten. Und die pathologisch-anatomischen Befunde am Versuchstier geben den Beweis, dass der Ursprung der Leukozytose in Zustandsänderung des Knochenmarks liegt.

Aus den Experimenten über den Antagonismus und aus übrigen Kontrollversuchen mit Na-haltigem Salze geht hervor, dass die Wirkung des Kochsalzes bei diesem Studium wesentlich auf die Wirkung des Na-Ions zurückzuführen ist.

Es ist interessant, das Blutbild bei der Kochsalzinjektion mit dem bei der parenteralen Proteinkörperzufuhr zu vergleichen. Das Blutbild bei der parenteralen Proteinzufuhr ist folgendes. Auf die initiale Leukopenie folgt eine länger dauernde Leukozytose, die in den nächsten Tagen abklingt und nunmehr wieder einer mehrere Tage dauernden Leukopenie (gegenüber dem Ausgangswert) Platz macht. Vollkommen parallel mit den Neutrophilen gehen die Monozyten. Auch die Lymphozyten zeigen ganz den gleichen Ablauf, aber in einem viel langsameren Tempo. Die obengenannte Blutbildsveränderung hat Ähnlichkeit mit meiner Blutbildsänderung durch die Injektion von 0.85% Kochsalzlösung.

Es ist wohl anzunehmen, dass in dem Prozesse der Blutgerinnung die Blutplättchen eine wichtige Rolle und zwar als Träger des Fibrinferments spielen (*Welch, Howell, Bordet, Derewenko, Gramer, Arnold, Schwalbe, Bürker*). Die Anwendung von Kochsalz per so für den Zweck der Blutstillung ist schon lange bekannt (*Sticker, v. Weismayer*). Die blutstillende Wirkung des Kochsalzes wurde dadurch erklärt, dass bei der oralen Darreichung des Kochsalzes durch den Reiz der Magenschleimhaut reflektorische Gefässerweiterung des Splanchnicusgebietes und dadurch die Ansammlung einer grösseren Blutmenge entsteht. Wegen dieser Kollapserscheinung treten Erniedrigung des Blutdruckes und Verlangsamung des Blutstroms im grossen Kreislauf und im Lungenkreislauf auf, dadurch wird die Blutgerinnung beschleunigt. *Grawitz* meint die Wirkung des Kochsalzes als Hämostaticum auf eine Eindickung des Blutes zurückführen zu sollen. *V. d. Velden* hat ausführliches Studium über die hämostyptische Wirkung der Bromide und Chloride an Menschen und Kaninchen ausgeführt. Er benutzte neben oraler oder subkutaner Darreichung auch die intravenöse Anwendung der Kochsalzlösung, um den Reiz des Magendarmkanals auszuschliessen. Er hat leichte Verminderung der Erythrozytenzahl nach der Kochsalzinjektion d. h. eine schwache Verdünnung des Blutes bemerkt. Er hat niemals Hämoglobin im Harn, hämotogenen Ikterus oder Hämoglobinämie gefunden. Aus diesen Resultaten hat er die Annahme ausgeschlossen, dass der Zerfall der Erythrozyten eine Beziehung zur Beschleunigung der Blutgerinnung habe. Er hat ferner eine grössere Zunahme der Leukozyten bei den Kaninchen als bei den Menschen durch die Injektion von konzentrierter Kochsalzlösung bemerkt. Durch sein Studium hat er die hämostyptische Wirkung

des Kochsalzes auf die Mobilisierung der Thrombokinase aus dem Gewebssaft in das Blut zurückgeführt, welche infolge der Veränderung der Blutkonzentration durch die Kochsalzinjektion stattfindet. Eine analogische Erklärung haben *Brücke* und *Nasse* gegeben, indem sie die Zunahme der Gerinnbarkeit nach der Blutentleerung als die Folge des Eindringens der Thrombokinase aus dem Gewebssaft in das Blut annahmen.

Aus meinem Ergebnis folgt, dass die Injektion von 10%iger Kochsalzlösung eine Vermehrung der Blutplättchen im Blute hervorruft. Man kann vermuten, dass diese Vermehrung der Blutplättchen eine wichtige Beziehung zur Gerinnbarkeit des Blutes habe. Die Zunahme der Blutplättchen nach der Injektion von 27.6% Na_2SO_4-Lösung stimmt mit der hämostyptischen Wirkung des Natriumsulfates überein, welche von *Reverdin* konstatiert wurde.

Die Injektion von 0.85% Kochsalzlösung an den immunisierten Kaninchen ruft auch hochgradige Leukozytose hervor. Nach dieser Leukozytose wurde die Vermehrung der Agglutinine und bakterizide Kraft im Blute beobachtet. Aus dieser Tatsache kann man vermuten, dass das Kochsalz durch den Reiz der blutbildenden Organe die Bildung und Fortschaffung der Immunkörper anregt. Ebenso ist von *Starkenstein* die Kochsalzlösung als Analogon der Proteinkörperwirkungen angegeben, welche die Antikörper und Resistenz im Tierversuch steigert. *K. Koike* hat die Einfluss der physiologische Kochsalzlösung u. s. w. auf die Hämolysine untersucht. Nach ihm hat Kationen eine wesentliche Beziehung zum Hämolysintiter und zwar wirkt K-Ion den Hämolysintiter erniedrigend, dagegen Na-Ion erhöhend.

Das Kochsalz übt mässig starken Einfluss auf das Blut und die hämatopoetischen Organe aus. Die vermehrte Ansammlung der pseudoesinophilen Zellen und die Hyperämie in dem Knochenmark können als eine Reaktion des Knochenmarks durch Kochsalzinjektion betrachtet werden. Und zwar können wir mit *Kaznelson* und anderen annehmen, dass es nach anfänglich bloss vermehrter Ausschwemmung zu einer Ausbreitung von parenchymatösem Gewebe des Knochenmarks über die normalerweise fetthaltigen Partien kommt.

Über die Entstehung der Erythrophagie ist mehrfach berichtet worden. Die Beziehungen der Phagozytose zur Chemotaxis sind vielfack noch unaufgeschärft und jedenfalls durchaus nicht einfach. *Hirschfeld* hat seine Beobachtung der Erythrophagie in den Kupfferschen Sternzellen der Leber bei perniziöser Anämie sowie nach der Injektion von Aqua destillata bei Ikterus gemacht. Er hat diese Erscheinung für die schädliche Wirkung der Aqua destillata gehalten. *E. M. Pearce* hat nach der Splenektomie des Hundes den Zerfall der Erythrozyten in den Reticuloendothelien der Lymphknoten und der Leber beobachtet. *G. Tizzoni* und *Fileti* sowie *Winogradow* haben auch Erythrophagie in den Lymphknoten nach Milzexstirpation beobachtet. *T. Nishikawa* und *T. Takagi* haben nach der Milzexstirpation eine Phagozytose roter Blutkörperchen durch die Kupfferschen Sternzellen und kompensatorischen Wucherung der Milzgewebe in der Leber

beobachtet, sie haben diese Veränderung als vikariierende Erscheinung für das Ausfallen der Milz angedeutet. Nach *Karsner*, *Bmirol* und *Bock* beginnt die Erythrophagozytose in dem Lymphknoten schon 3 Stunden nach der Injektion von hämolytischem Serum, erreicht zwischen 12–24 Stunden nach der Injektion das Maximum und wird nach 48 Stunden dort die Pigmentansiedelung als Rest der Erythrophagie gesehen. *Dürk* fand am dritten Tage nach der chirurgischen Operation am höchsten ausgebildete Erythrophagozytose, und sah die Erythrozyten im weiteren Verlauf sich in Hämosiderin umwandeln. Im allgemeinen können wir in den Reticuloendothelien der Lymphknoten des normalen Kaninchens altes Blutpigment sowie ganz vereinzelt spärliche phagozytierte Erythrozyten sehen. *Kretz* hat durch intravenöse Injektion von distilliertem Wasser, Curare, Pepton beim Hund, Huhn oder Kröte, Diapedese der geschädigten Erythrozyten und Phagozytose derselben durch Leberzellen erziehlt. Nach *Roessle* beginnt die Aufnahme roter Blutkörperchen in Parenchymzellen (Leber, Pankreas, Niere etc.) mit infektiös-toxischer Schädigung der Kapillaren und mit hämorrhagischem Oedem, das zum Zerfall der Erythrozyten führt. *Y. Kon* hat durch subkutane Injektion der Salicylsauresnatronlösung Erythrophagie in den Retikuloendothelien der Leber sowie der Milz geschehen gesehen, und daraus kam er der Meinung dass Erythrophagie nicht nur mit geschädigten Erythrozyten in solchen Körperzellen stattfindet, wie es bisher allgemein anerkannt ist, sondern mit *Kyes* auch aktiv für die Lieferung des wichtigen Pigments zur Gallenbildung von gewisser Bedeutung sei. Durch die Untersuchung über die Erythrophagie in den weissen Blutzellen zog *H. Uchida* den Schluss, dass es wenigstens in seinen Experimenten keine Erythrophagie entsteht, wenn die Erythrozyten weder physikalisch chemisch noch von den sogenannten spezifischen Hämotropin beeinflusst werden.

Ganz kürzlich, (Nov. 1922) hat *Rautmann* eine neue Anschauung über die Milzfunktion angegeben. Seiner Methode gemäss habe ich folgende Versuche gemacht; durch intravenöse Injektion von 10% NaCl-lösung, 2 ccm pro kilo, blieb das Verhältnis der Erythrozytenzahl zwischen Milzarterienblut und Milzvenenblut fast wie normal; es zeigte sich keine auffallende Abnahme der Erythrozytenzahl des Milzvenenblutes im Vergleich zum Milzarterienblut.

Aus meinem histologischen Befunde, aus dem Ausbleiben der Resistenzänderung der Erythrozyten und aus der oben erwähnten Resultaten der Milzvenenblutuntersuchung kann man vermuten, dass bei meinen Versuchen mit Kochsalzinjektion die Zustandsänderung der Kapillarenwand und die aktivierte Tätigkeit der Reticuloendothelien eine wesenlichere Rolle für die Erythrophagozytose als die Abschwächung der Vitalität der Erythrozyten selbst spiele. Die Auffassung *Kaznelson*'s erscheint mir wahrscheinlich, dass die nach Typhusvakzininjektionen sowie nach parenteraler Verabreichung von Proteinkörper auftretende Milzschwellung vielleicht unspezifisch sei, ebenso wie die Veränderungen der Lymphknoten, weil wir die gleichen Veränderungen gerade auch bei der Kochsalzinjektion beobachten können. Das Auftreten von Erythrophagozytose nach der Bluttrans-

fusion wurde von *Hunter* und *Gabbi* berichtet, dieses stimmt mit meinem Experiment überein. Was die Erythrophagie selbst betrifft, ist es hier ausser Frage ob es eigentlichen Blutlymphknoten handelt oder nicht.

Resumé.

1. Das Chlornatrium ist keine indifferente Substanz, auch nicht in seiner isotonischen Lösung, sondern hat eine Reizwirkung auf das Blut und die hämatopoetischen Organe. Die Wirkung des Kochsalzes bei meinem Studium ist wesentlich auf die Wirkung des Na-Ions zurückzuführen.

2. Die intravenöse Injektion von 0.85% Chlornatriumlösung, 2 ccm pro Kilo des Körpergewichtes, ruft auffallende Leukozytose hervor.

3. Die Zunahme der Leukozyten betrifft hauptsächlich die pseudoeosinophilen Leukozyten, während der Lymphozytenwert nur eine minimale Schwankung erfährt.

4. Die Injektion von Aqua destillata sowie 0.3% NaCl-Lösung verursacht keine besondere Blutbildsschwankung.

5. Die Injektionen von 0.6%, 0.9%, 1.7% sowie 3.4% NzCl-Lösung zeigen sogut gleiches Resultat wie 0.85% NaCl-Lösung.

6. Die Injektionen von 10.0%, 25.0% sowie 26.4% NaCl-Lösung erzeugen Leukozytose von etwas geringerem Grad. Dabei wird eine leichte Verminderung der Erythrozytenzahl und des Hämoglobingehaltes beobachtet.

7. Die Leukozytose zeigt durch Kochsalzinfusion gleiches Verhalten im peripheren Blut und im Lebervenenblut.

8. Durch die Injektion von 14.9% NaBr-Lösung sowie von 2.34% Na_2SO_4-Lösung werden ähnliche Resultate wie bei 0.85% NaCl-Lösung erzielt. Die Injektion von schwach alkalisierten Salzgemisch NaCl 0.85% plus $NaHCO_3$ 0.01% ruft ebenso auffallende Leukozytose hervor.

9. Das Salzgemisch NaCl 0.85% plus $BaCl_2$ 0.04% erzeugt keine besondere Leukozytenschwankung, während die 0.04% $BaCl_2$ Lösung eine auffallende Leukozytose verursacht.

10. Die Injektionen der folgenden Salzgemische rufen hochgradige Leukozytose hervor:

NaCl 0.85%	NaCl 0.85%
$CaCl_2$ 0.02%	$CaCl_2$ 0.06%
	KCl 0.06%
NaCl 0.85%	NaCl 0.85%
$CaCl_2$ 0.02%	$CaCl_2$ 0.1%
KCl 0.02%	KCl 0.1%

11. Durch die Injektion von 10% NaCl-Lösung, 1 ccm pro Kilo, wird die doppelte bis dreifache Vermehrung der Blutplättchen hervorgerufen. Die Vermehrung beginnt schon 30 Minuten nach der Injektion und dauert 5 bis 6 Stunden

lang. Dabei vermehren sich die Leukozyten durchschnittlich ums doppelte, während die Erythrozytenzahl und der Hämoglobingehalt sich in geringerem Grade vermindern. Die Resistenz der Erythrozyten 10 m, 30 m, 1 Std, 2 Std, 4,5, und 6 Stunden nach der Injektion von 10% NaCl-Lösung zeigt keine Veränderung.

12. Die Injektion von 27.6% Na_2SO_4 Lösung zeigt eine starke Zunahme der Blutplättchen wie bei 10% NaCl-Lösung. Die Injektion von 50% Traubenzuckerlösung erzeugt 55–70% Vermehrung der Blutplättchen.

13. Durch die intravenöse Injektion von 0.85% NaCl-Lösung, 5 ccm pro Kilo, bei den immunisierten Kaninchen wird hochgradige Leukozytose hervorgerufen. Nach dieser Leukozytose wird die Zunahme der Agglutinine und der bakteriziden Kraft beobachtet.

14. Das Knochenmark des Versuchstieres ist blutreich und ödematös und vermehrte Ansammlung der pseudoesinophilen Zellen wird bemerkt.

15. Nach wiederholten Injektionen von 10% NaCl-Lösung wird die Vermehrung der Megakaryozyten im Knochenmark und die vermehrte Embolie von Megakaryozyten in den Alveolensepten der Lunge beobachtet.

16. Bei der Injektion von Kochsalzlösung wird hochgradige Erythrophagie in den Hals-Inguinal- und Retrosternallymphknoten, sowie auch nur in geringerem Grad in den Mesenteriallymphknoten beobachtet.

17. Nach wiederholten Injektionen von 10% NaCl-Lösung wird eine bedeutende Hämosiderinablagerung in den Lymphknoten, in der Milz, im Knochenmark, oft auch in den Leber gefunden.

18. Bei der Injektion von 10% NaCl-Lösung sowie bei der Auto- und Iso-Blut Transfusion werden neben den normalen eosinophilen Zellen zahlreiche atypische eosinophile Zellen mit stäbchenförmigen Granula in der Milz und in den Lymphknoten beobachtet.

Zum Schlusse fühle ich mich verpflichtet, meinem hochverehrten Lehrer, Herrn Professor Dr. *M. Nagayo*, für seine freundliche Anregung und Anleitung bei der Ausführung dieser Arbeit meinen herzlichsten Dank auszusprechen. Herr Prof. Dr. *H. Hayashi* und Herr Prof. Dr. *Y. Miyagawa* haben mich bei dieser Arbeit mit vielfachen Ratschlägen unterstützt, wofür ich auch diesen Herren zu aufrichtigem Dank verpflichtet bin.

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Erklarung der Abbildungen.

Fig. 1. Erythrophagie im Sinus des Halslymphknoten. Kaninchen Nr. 220 0.85% NaCl-Lösung 5.0 ccm pro Kilo dreimal injiziert. Zeiss Okul. 4, Obj. Immersion.
Fig. 2. Erythrophagie im Sinus des Halslymphknoten. Kaninchen Nr. 201 10.0% NaCl-Lösung 1 ccm pro Kilo zweimal injiziert. Zeiss Okul. 2, Obj. DD.
Fig. 3. Leukozytenstauung im Knochenmark (Femur) Kaninchen Nr. 87 10.0% NaCl-Lösung 1 ccm pro Kilo viermal injiziert. Zeiss Okul. 5, Obj. Immersion.

Fig. 1.

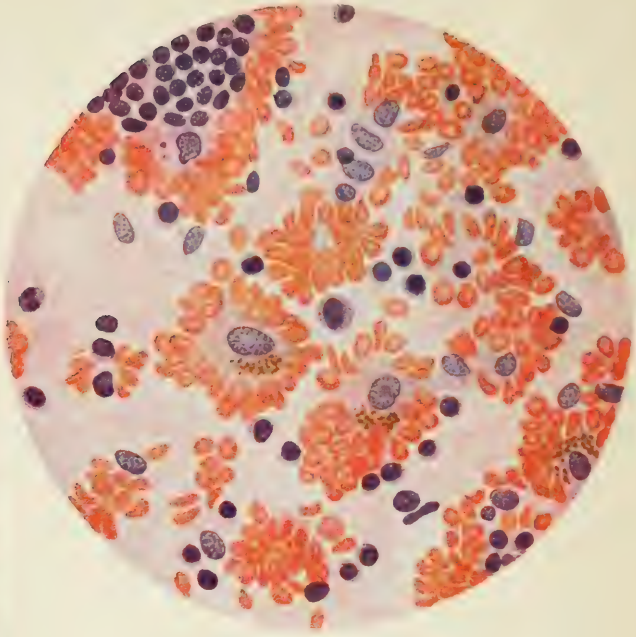


Fig. 2.

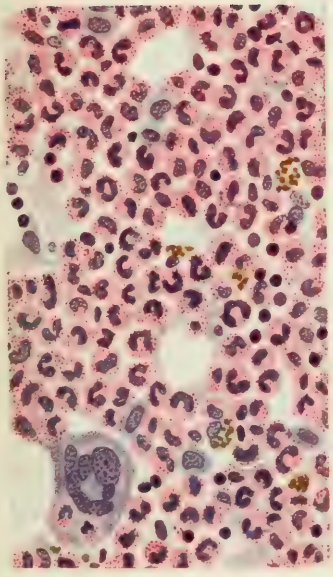
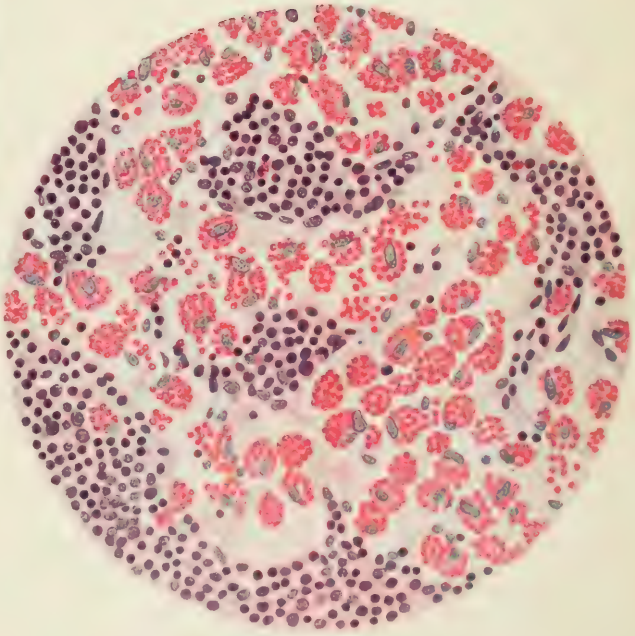


Fig. 3.



(Dr. S. Takamura : Beiträge zur Kenntniss der biologischen wirkung des Chlornitriums auf das Blut und die hämotopoetischen Organe.)

The Influence of some Components of old Tuberculin and Tubercle Bacillus on the Progress of experimental Tuberculosis.

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Foreword.

The infectious diseases which may take the chronic course are generally very much alike in their state of immunity, although the virus of each disease possesses very different qualities.

Thus, the state of immunity of protozoa diseases which belong to what Schilling called labile infection, and of anthrax and glander is compared and discussed with that of tuberculosis by many scholars.

With regard to the experimental artificial immunity of tuberculosis, the investigation which has been made up to this day has given us the following generally admitted principles.

1. Using the dead bacillus as immuno-antigen only generates antibodies in the serum and seldom immunizes the animal.

2. But when a healthy animal is made to be infected by the weak virulent living bacillus, it acquires a certain degree of immunity against reinfection of the strong virulent bacillus, (first discovered by *Koch*.)

3. When the human type bacillus is inoculated to the cattle, the disease is localized. But it shows remarkable immunity against reinfection of the strong bovine type bacillus untill the disease by human typ bacillus is entirely cured. (first reported by Behring in 1901. Nobel Lecture.)

4. The so-colled antibody (complementbinding anti-body, opsonine, tropine, agglutinine, precipitine) does not necessarily accompany with the immunity of animals. But when an animal shows the state of immunity, the reactive inflamation occurs regularly against the inoculation of the living bacillus. (As to whether the cause of immunity is to be ascribed to this special reaction itself, or it is simply a secondary reaction and has nothing to do with the true cause of immunity, opinions are divergent.)

5. Although the tuberculous animal is immune against reinfection, its own tuberculous focus continues very long and may make a gradual progress.

My investigation concerns the vaccine component which has a definite influence on the progress of tuberculosis caused by inoculation of tuberculous bacillus to the guinea pig. It is my belief that an interesting result was obtained The author publishes it with a hope that it might give some light to the artificial immunotherapy of tuberculosis.

Method of experiment.

The bacillus is the human type (name *Aoyama* bacillus). It has been preserved for several years, being made to pass the guinea pig body twice a year. I took the bacillus moss cultivated on 4% glycerin bouillon at about 10 days growth when it is most vigorous. After grinding it well in an agate morter, and then diluting it in saline water, I inoculated it to the hypoderma of guinea pigs. In inoculating tuberculous bacillus to the guinea pig, more than 1/10,000,000 mg. of bacillus is usually needed to cause an equal state of infection. In less quantity, there will be greatly varying degrees of infection according to the individuality of the animal. In order to make my experiment certain, I used comparatively larger quantity, i. g. more than 1/100,000 mg. of bacillus.

Method of inoculation.

There are various methods of inoculating tuberculosis bacillus to the guinea pig. The intraperitoneal injection makes infection more easily, but a little increase of quantity of bacillus infected would cause the inflamation of the peritoneum and adhesion of the infected part, and makes consequently the unequall state of infection among different animals. The intraveinous injection will secure comparatively equall state of infection. But it is attended with the disadvantege that we can not see the state of progress of tuberculosis. Therefore following the method of Römer which was used to know the resistance against reinfection, I made the subcutaneous inoculation of the descending quantity of bacillus. This method enables us not only to know the pathological change of the inoculated part, and the state of progress but also gives us the advantage of allowing us to make the comparative elimination of the difference of resistance of different individual by comparing the difference of quantity of bacillus, and thus to lessen the number of animals to be experimented on. In experiment, I plucked off the hairs with my fingers from that part of the abdominal wall on which the inoculation is to be made, and washed it with alcohol. Then I applied the injector to a point about $\frac{1}{2}$ cm. above the navel and made an effective subcutaneous injection at about 1 cm. above the navel.

The state of the progress of tuberculosis of this guinea pigs was in agreement with so called law of Cornet which is known by a classical appellation, "Localizations gesetz". The tuberculosis was mainly of lymph system. I selected such guinea pigs which were in age and weight generally alike to those used for control, and I selected the male only, for pregnancy in guinea pig

remarkably accelerates tuberculosis. The intracutaneous tuberculine reaction (Römer) of selected animals was negative.

Method of Observation.

I closely observed the states of induration, abscess, and ulcer which were gradually formed in the part when bacillus is inoculated every day or at the interval of two or three days, and recorded the result. In the case of obduction, I examined whether the inoculated part had abscess only, or had formed an ulcer, or the trace is made indistinct by forming a scar. Then I examined the size and the degree of caseation of lymph gland. For the examination of the internal organ, I made the external macroscopic observations of the number and size of the tuberculous node in the organ, and compared them with the contrast arranged on the sametable. Especially notice was put on the examination of spleen. By histological observation I put especially notice on the state of cell destruction (caryolexis, necrosis, caseatin & haemorrhagy.)

Chapter I.

Experiments in which the component of old tuberculin is used as vaccine material.

1. Old tuberculin.

The moss of the bacillus, two months cultivation on 4% glycerin buillon is sprinkled, and is made to fall entirely. After a few weeks it is killed by heating at 70°–90°C. for $\frac{1}{2}$ hour. Then it is slowly filtrated with filter paper. The filtrate is condensed to 1/10 of the original volume by heating at about 60°–80°C. and is stored in an ice chest.

2. The method of preparing the digested tuberculin.

To old tuberculin is added three fold quantity of saturated ammon sulphate. The mixture is left 24 hours in room temperature (cold). The sediment is collected in filter paper and freed from humidity. It is then dissolved in distilled water. (the quantity of which being one half of the original tuberculin.) The solution is mixed with the same quantity of 2% pancreatin distilled water, and slightly alcalinized and is digested for $\frac{1}{2}$–2 hours at 38°C. After the destruction of pancreatin by heating it for $\frac{1}{2}$ hour in 60°–63°C., it is mixed finally with 0.3% carbonic acid, and stored in an ice chest.

3. Digested bouillon.

This is prepared by the same method as the preceding one ; only, condenced 4% glycerin bouillon is used instead of old tuberculin.

4. The digested alcohol soluble substance of old tuberculin is prepared in the following way.

To aforesaid old tuberculin is added 3 fold quantity of 95% alcohol. The clear upper portion of the liquid is collected and filtrated untill it becomes perfectly transparent. When this is vaporized, then remains syrup like residuum to which

distilled water is added untill the volume of the mixture becomes one half of the original tuberculin. It is then mixed with 2% pancreatin distilled water, and alkalinized and digested in water bath maintained at 38°C. for 1–3 hours. After the component which produces the tuberculin skin reaction is destroyed, the liquid is heated at 60°–63°C. for $\frac{1}{2}$ hour. (Destroyance of reactable substance in tuberculin by pancreatin was examined by pirquet test and injection in tuberculous guinea pigs.)

The first Experiment.

Fifteen large (500–600 gm) guinea pigs were selected and divided into 3 groups (A, B, C). To each of them tubercle bacillus was inoculated in the desending quantity 1 mg, $\frac{1}{10}$ mg, $\frac{1}{100}$ mg, $\frac{1}{1000}$ mg, $\frac{1}{10000}$ mg respectively on 30/X and after 36 days (on 41/XII) the guinea pigs were killed and dissected. For A group the vaccination was began with 1 c.c. of digested tuberculin and for B group with 0.3 c.c. of old tuberculin. As a control, animals of C group were observed without vaccination.

The dates of vaccination....30/X, 31, 1/XI, 23, 4, 5, 6, 8, 9, 10, 12, 15, 17, 19, 22.

The most remarkable differences were seen;

(1) In the state of the inoculated part.

In case when the digested tuberculin was injected, for animals which was infected with the quantity of bacillus less than 1/100 mg., the formation of abscess was seen up to certain point: but beyond this point, the progress was stopped or there was a slight decrease. And these ended without forming ulcer. In contrast experiment, there was always the formation of ulcer. In case when old tuberculin was injected, there was formation of ulcer just as in the case of contrast experiment, but, when more than 1/100 mg of baccillus was inoculated, ulcer therby formed was exceedingly large. It should be noted that the phenomenon of inhibition of the progress of abscess or ulcer in the inoculated part is usually seen when an animal under test is debilitated. In such a case, although there is no hypertrophy of lymph gland and spleen, the caseous degeneration of lymph gland is remarkable, and usually the number of the tuberculous nodes of spleen are very great. In other words, the extent of the formation of ulcer in the skin does not necessarily correspond with the extent of tuberculosis on internal organs. The cause of it may be imagined to be weaking of the reacting power. In this experiment between those to which tuberculin was injected and those to which digested tuberculin was injected, there was not much difference in the decrease of the weight of body and the exhaustion of physical strength. But compared to those in the contrast, they all showed a remarkable decrease of weight. Hence we should not think that the absence of ulcer in the case of the injection of the digested tuberculin was the result of the decrease of physical strength only. We ought to consider other and more constant influence also. Again, the fact that in the case of tuberculin injection,

there was an acceleration of the formation of ulcer, in spite of a similar decrease of the weight of body, should be explained as the result of some influence of tuberculin injection.

(2) Of the anatomical observation, the most remarkable is the appearance of the spleen. In those to which digested tuberculin was injected, for the animals which is injected with the quantity of 1/100 mg tuberculous bacillus, it is not certain that one could recognize macroscopically the presence of tuberculous nodes. For less quantity, no apparent and macroscopic recognition of tuberculous nodes is possible, and the spleen appears perfectly smooth. But in the contrast, even for 1/10000 mg of bacillus, the tuberculous focus of high grade could be easily recognized. In the case of tuberculin injection, for the quantity of bacillus less than 1/100 mg, the macroscopic recognisation of the nodes was possible, but they were noticably smaller in number than for the same quantity of bacillus in the contrast and destruction of tissue was minimal.

In other organs of animals which were infected with less than 1/100 mg tuberculous bacillus vaccinated with the digested tuberculin, the affection was apparently slighter than the contrast group. But in those to which tuberculin was injected, compared with the contrast, the difference was not so remarkable as the former, and the state of caseous degeneration of lymph gland was even more remarkable.

The second experiment.

In the first experiment, we have observed that the digested tuberculin (or the tuberculin of which the precipitive component by ammon sulphate digested by pancreatin and whose reacting component was destroyed) showed a remarkable inhibitory action on the formation of the tuberculous nodes and destroying action of it on tissue or cells which was observed especially in spleen; but when tuberculin was injected, this action became slightly weaker and more over, the caseous degeneration of the lymph gland and the ulcer of the skin were even more remarkable than in the cases of the contrast. Then the inhibitory action on the progress of the tuberculous nodes is certrainly due not to the injection of the substance which causes the tuberculin reaction: but it must be ascribed to the action of some substance contained in it, or the state of the digested tuberculin. But as the matter of fact, even in the digested tuberculin there is found as small quantity of reacting component. So we should carefully consider whether, the amount of this component which manifests the tuberculin reaction in the digested tuberculin is just what is needed to rouse the inhibitory action on the progress of the tuberculosis; and whether, while too much presence of the reacting component in the old tuberculin weakens the inhibitory action on the progress of tuberculosis, (In fact tuberculin injnction made the animals anergic).

These problems but be solved now. So solve them, in the second experiment, the digested tuberculin and the salt water tuberculin solution were so made as to contain the same amount of the substance which produces the tuberculin skin-

reaction. On one hand, the digested tuberculin was so prepared as to produce the same amount of the skin reaction as approximately 1% tuberculin diluent. On the other hand, tuberculin was diluted to 5% in salt water. Therefor 0.1 c.c of tuberculin salt water solution and 0.5 c.c. of digested tuberculin must contain the same quantity of reactive substance.

Remark. The comparison of tuberculin reaction was made by performing Pirquet reaction on the forearms of the writer and his colleague.

24 guineapigs of 200–300 gramms of the weight were selected and divided into 3 gloups. 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} mg. of tuberculous bacillus were inoculated on 4/III to every two pigs of each 3 group. Then the animals of the first group were injected with 0.1 cc of 5% old tuberculin once a day from 4/III to 9/III; and 0.2 cc each on 10, 11, 12, 14, 15, 16, 19, 21, and 23/III.

To those of the second group, 0.5 cc and 1 cc of the digested tuberculin were administrated in the similar way as the first group.

The 3rd group remained untouched.

The result of the second experiment, like that of the first experiment, has enabled us to recognize clearly the inhibitory action on the progress of tuberculosis, when the digested tuberculin was injected. But in the case of the injection of the salt solution of tuberkulin, which contains the same quantity of reacting component as the digested tuberculin, it was difficult to recognize this action, and the caseous degeneration was even more marked than control. In this experiment, even in the case of the injection of the digested tuberculin, there was only one guinea pig, nomely, No. 14, (infected with 10–3 mg of T. B) in which the formation of ulcer in the inoculated part was checked. This result seems to run counter with that of the first experiment. But at the same time, I have recognized that there was unmistakable delay of the formation of ulcer in other cases. Thus the following conclusion could be made. From the results of the first and the second experiments, it is clear that the inhibition for the formation of the tuberculous focus was due not to the injection of the substance which produces the so colled tuberculin reaction but to the action of other substance contained in the digested tuberculin.

As stated before, the sediments through ammon sulphate can be digested by pancreatin and there-by be separated from the component which produces the tuberculin reaction. (It is not clear whether this inhibitory action was newly aroused or simply increased by the digestion of tuberculin, or it had already existed in it and now shown its activity as the result of the destruction of the substance which produces the tuberculin reaction. Only in the first experiment where a large quntity of old tuberculin was used, the number of tuberculous nodes was small in the spleen in spite of the remarkable formation of ulcer at the inoculated part. This fact remind us that this activity reside in old tuberculin beforhand.

Here we have to note that there must be some reason for the fact that,

although the small quantity of tuberculin has a tendency to augment the causeous degeneration and ulcer, it makes more increase in the weight of body than in the cases of the control and of the injection of the digested tuberculin.

The third experiment.

In the first and second experiments, the digested tuberculin was injected after the inoculation of tubercle bacillus. In this experiment, however, the injection was made before and after the inoculation of tuberculous bacillus on 16 test animals. But its result, just as those of the first and second experiments, has proven the inhibition of the formation of the tuberculous nodes. Unlike the first experiment, the formation of abscess or ulcer was not entirely checked in the present experiment but the formation of the latter was much slower than control.

The fourth experiment.

The action of the digested tuberculin in the preceding experiment may perhaps have been due to the bouillon component. This experiment was directed to make that point clear. Instead of tuberculin, glycerine bouillon was taken up and was concentrated to 1/10 of its volume. Three fold quantity of ammon sulphate was added to it, and the resulting sediments being collected and dissolved in one half of the original quantity of the distilled water, was digested by pancreatin and made into vaccine. The injection of this liquid was made in the same way as in the third experiment.

The result, enables us to see that it had not only no inhibitory action on the formation of the tuberculous nodes, but it has augmented the tuberculous disease in the spleen in a higher degree than in the contrast. We conclude, therefore, that the inhibitory action of the digested tuberculin on the formation of tubercles does not depend upon the digested product of the bouillor component contained in it, and that it ought to have a special relation to the tubercle bacillus itself.

The fifth experiment.

For this experiment, 15 guinea pigs were selected. To 3 of A group, digested alcohol soluble component of old tuberculin was injected as vaccine. In order to make injection to 6 of B group, a vaccine was prepared in the following way. 20 gr tubercle bacillus which was sterilized in 60°C. for 1 hour, was mixed with 100 cc of 50% glycerine salt water and was put in an incubator for 2 weeks. It was then filtrated through *Reichert* filter. The filtrate clearly showed *Pirquet* reaction. To this, the equall quantity of 2% pancreatin water was added, and the mixture was digested in 38°C. for 2 hours, so that *Pirquet* reaction entirely disappeared.

To 6 C group which were used for the cotrast, nothing was done.

The result is shown in the table.

In those of A group to which less than 1/1000 mg. of bacillus was inoculated, small abscess was formed ; but it did not breake and form ulcer. In anatomical examination, none in A group was seen macroscopically to have the nodes in the

spleen. But in B group and the contrast, on the contrary, the presence of tubercles was ascertained macroscopically. In this experiment, only three were used for A group. But the difference was so remarkable that we had to account for it not refering it to the difference of apriori resistance of the individual only, but to some influence of vaccine. In other words, we have to admit that there must be the inhibitory action on progress of tuberculosis in alcohol soluble component of tuberculin also. But in the glycerin infusion of tubercle bacillus which is digested by pancreatin, there is no such action.

Chapter II.

In this experiment the bacillus body was used instead of tuberculin.

1. The method of preparing vaccine material.

The young tubercle bacillus was well dried and ground in an agate mill continually for three weeks. The ground bacillus was infused in Ether by room temperature for several days and then it was filtrated through *Reichert* filter untill it became perfectly clear. This fluid was desiccated in relatively low temperature and was infused with 95% alcohol for 30 minutes. It was then centrifugalized. The sediments thus made was desiccated again and mixed with 10% olive oil. This was assumed to be ether soluble alcohol nonsoluble component.

2. The upper clear part of beforesaid centrifugalized liquid was desiccated and an oil shaped lump was procured from it. An ether infusion described in 3 was desiccated. The two were mixed with each other, which was again mixed with olive oil at the rate of 2%. This was assumed to be ether-soluble alcohol-soluble component.

3. The above described ground bacillus was put in to alcohol in room temperature for over 48 hours and then filtrated though *Reichert* filter. A perfect clear liquid was collected from it and desiccated at relatively low temperature. Ether was poured on it. After 20 minutes, ether was removed and the insoluble component was collected which was mixed with olive oil at the rate of 1%. This was assumed to be ether-non-soluble alcohol-soluble component. That which was dissolved in ether must be assumed as an alcohol-soluble ether-soluble component and the last one was mixed to the 2nd component.

In this way, three kinds of components were prepared and experimented with as vaccine.

The method of experiment.

24 guinea pigs of middle size were selected and divided into 4 groups (A, B, C, D). To every two animals of each group a descending amount (10^{-2}, 10^{-3}, and 10^{-4} mg) of the tuberculous facillus was inoculated on 21/VI.

Animals of A group were treated with ether & alcohol soluble substance of tuberculous bacillus (2% in olive oil), those of B group with ether soluble but alcohol insoluble substance (10% in olive oil) and C group with ether insoluble

but alcohol soluble substance. (1% in olive oil).

D. Group were investigated as control. Vaccinations were carried out with 0.1 cc of each vaccines on 21/VI, 22, 23, 24, 25, 26, 27, 29, 30, I/VII, 2, 3, 4, 6, 7. Animals were killed after 36 days (on 26/VII) and examined.

Generally speaking, the inhibitory action on the progress of tuberculosis is most remarkable in ether and alcohol-soluble component (the second component). This action is also recognized in ether insoluble, alcohol soluble component (the third component). But compared with the contrast, the 1st component (ether soluble alcohol insoluble component) showed almost no inhibitory action on the progress of the tuberculous nodes, although the weight of the test animal was remarkably increased by administrating this component.

Chapter III.

The tuberculin sediment through ammon sulphate, the tuberculin sediment through alcohol and the alcohol soluble substance of tuberculin were digested by pancreatin and their activities were tested with the view of finding which of them is most effective.

They were prepared in the same way as that which was described under the preparation of vaccine material. Only, care was taken that in any manipulation of preparing tuberculin for this experiment, no higher heat than 65°C. was not given lest the effective component might be destroyed. The method of experiment with the animal, and the method of observation were the same as those in the preceding chapter. But the way of vaccine injection was different. As shown in the table, before inoculating tubercle bacillus, the subcutaneous injection of 1 c.c. of vaccine was made four times at the interval of a day or two. After inoculation for first eight times, 0.5 c.c. was injected at the interval of one or two days. After that, every week or every ten days, the injection was made.

The animal was killed on the 50th day of inoculation and dissected.

Now the tuberculin sediment through ammon-sulphate which is digested is called A vaccine. The digested alcohol sediment is called B vaccine. The alcohol soluble substance of tuberculin which is digested is called C vaccine.

a. When the animals were treated with C vaccine, the formation of abscess or ulcer in the focus to which the bacillus was inoculated became exceedingly slow thus in the animals to which 1/1000 mg of the bacillus was inoculated, for the first time, abscess was broken on the 45th day and ulcer was formed. When A vaccine was applied the formation of ulcer was not checked so remakably as in the experiment described in the first chapter. But here ulcer formed the scar better than in any other cases. Some scars were first large enough to be visible and some were scarcely visible.

The control animal, on the other hand, all formed ulcer within 20 days.

b. In the animals which were treated with A and C vaccines, the two

inguinal lymph glands once underwent enlargement. But soon they contracted and softened so that they were not felt even when touched.

It is true even in the control animal, the inguinal lymph glands which once enlarged and stiffened, were generally reduced evidently after 40 days. But the change was not so remarkable as in the cases when treated with A and C vaccines.

c. The anatomical observation is shown in the table. In those animals which were treated with C vaccine the generalization of tuberculosis was most effectively checked, and there was only one animal in which the unmistakable tuberculous focus was formed in the spleen. In all others there was the enlargement of lymph follicles only, and no undoubted formation of the T.b. node was recognized.

A vaccine was next to it in effectiveness to check the generalization of tuberculosis. B vaccine did not show much effectiveness, except that in lessened some what the cheesy degeneration than the control animals.

Thus the result of the comparison of the inhibitory action of three kinds of vaccine on generalization of tuberculosis may be expressed in the following formula.

$$C \rangle\ A \rangle\ B \rangle\!\!/\ \text{control animal.}$$

d. appendix. C vaccine or the digested alcohol soluble substance in tuberculin did not act as antign for the complement fixation with the serum of tuberculous patients in which the presence of complement fixing antibody was decidedly shown by using *Müller*'s antigen. Again C vaccine dose not make complement fixation with the serum of a rabbit which has been treated with the same vaccine.

Conclusion.

1. The successive injection of a large quantity of old tuberculin to tuberculous guinea-pigs inhibited more or less the generalization of tuberculosis. But the ulcer in the inoculated part of the virus was more marked than in the control animals.

2. When tuberculin sediment precepitated by ammon sulphate and the alcohol-soluble substance in tuberculin which were digested by pancreatin and from which the substance producing the tuberculin reaction was removed, were used as vaccine, the formation of the ulcer in situs where the bacillus was inoculated, was slackened or hindered. Also generalization of tuberculosis, as well as the destructive action of tubercle bacillus on the tissue, (caryolexis, necrosis and cheesy degeneration) were effectively checked.

3. Very small quantity of tuberculin, when it succesively vaccinated, can accelerates the generalization of tuberculosis and the cell destructing action in lymph glands.

4. When the sediment of glycerin bouillon with ammon sulphate, which was digested, was used as vaccine, no inhibition of generalization of tuberculosis was recognized.

5. Thus, the following general conclusion may be drawn from the fact above mentioned. In tuberculin used as vaccine, we may be able to assume the presence of two kinds of substances; i. e, the one which hinders the generalization of tuberculosis and destructive action of tuberculous bacillus on the tissue and the ather one which can accelerates the tuberculosis. The active substance which mainfest tuberculin reaction appear to accelerates the progress of the disease, when it is used continuary as vaccin. This substance can be removed by the digestion with pancreatin.

6. When three kinds of digested vaccines by panereatin the tuberculin sediment obtained by the addition of ammon sulphate,(1) the alcohol insoluble portion of tuberculin,(2) and the alcohol-soluble substances of tubereulin,(3) are compared one another, it is found that the alcohol soluble substance is the strongest to demonstrate inhibitory action on experimentel tuberculosis, the ammon sulphate sediment is next to it, and the alcohol sediment is least effective, where the action of the vaccine can be scarsely noticed.

7. The alcohol extract of the powdered tubercle bacillus has the inhibitory action on generalization of tuberculosis, and on the destructive action of tubercle bacillus on the tissues. On the other hand, the alcohol- ethersoluble substance of tuberculous bacillus inhibited the similar action. But the alcohol insoluble, ether soluble substance, which can be obtained in the largest quantity from tubercle bacillus, are devoid of this property.

8. In the slight infection of tuberculosis, an enlargement of lymph follicle of the spleen is always demonstrated. But in the severer cases entirely disappearance of follicles can be seen sometimes.

9. The digested alcohol soluble substance of old tuberculin cannat act as antigens for the complement fixation either with the serum of tuberculous patient or those obtained from immunized rabbit.

10. The alcohol soluble substance of old tuberculin digested with pancreatin manifested the most remarkable inhibitory action upon the progress of experimental tuberculosis on guinea pigs and the serum of test animals thus treated contained the least amount of complement fixing antibody for emulsion of the tuberculous bacillus (Müller's antigen.)

Discussion and Appendix.

There is no definite opinion, to day, as to whether vaccine therapy and vaccine prophylaxis are different in the principle of the production of effect or no. But as the states of the production of their reaction or effect are, as the matter of fact, very different, we can not do otherwise than to investigate the two

separately. The same holds true of the vaccination against tuberculosis. It is exceedingly difficult to immunize the healthy guinea pig by using tuberculin or other dead material. But when tuberculin therapy is applied to tuberculous guinea pigs there is a definite effect. The experiments made by *Pfuhl* and *Kitasato* were the beginings of this kind of investigation. By them it has been proved that the experimental tuberculin therapy increases the resistance against tuberculosis, and manifests undoubted curative tendency in the tuberculous animal, and in other cases, it greatly prolonged the life of the animal compared to the control animal. *Pfuhl* tells us that a small amount of tuberculin injection would not bring the curative tendency; and in order to make it effective, more than a fixed quantity should be used. He used sometimes so much as 0.5 c.c. every other day. And from the fact that tuberculin does not react at all and has no prophylactic effect on a healthy animal, and when tuberculin therapy is effective, it is always attended with the reaction, he concluded that the cause of cure must be sought in the reaction itself due to tuberculin. This idea coincides in many respects, with the clinical experiences of many authers, and is still regarded as a weighty view. But there is none yet who has demonstrated that which is effective is the reaction itself of the substance in tuberculin which produces so called tuberculin reaction. My investigation has enabled me to infer that there are two kinds of components in tuberculin from the influence of the successive injections of tuberclin on the progress of tuberculosis. The first component is that which slightly accelerates the progress of tuberculosis, and contains substance which produces the tuberculin reaction and the bouillon substance. The second component checks the progress of tuberculosis. It can be thought to be a substance which does not precipitate by alcohol and can stand pancreatin digestion. This fact seems to negate the idea that the tuberculin reaction itself is the source of immunity. But I do not think such an inference is necessary. The healthy animal does not react to tuberculin and also is not made immune against tubercle bacillus by tuberculin. But the tuberculous animal reacts to tuberculin, and it increases the resistance or can be cured by tuberculin. So long as such relation exists, the hypothesis that the tuberculin reaction or stimulation has a definite relation to the curative effect has a strong reason. Only I believe there is room to imagine the presence of both necessary and unnecessary curative processes in the stimulation or reetion (in the term "reaction" are included the inflamatory reaction, the serological antibody production, and the generation of other bodies still unknown to us), of tuberculin on the tuberculous animal.

If my work is criticized from this view point, my so called second component may be regarded as an aggregate of necessary stimulants. In my experiments, the first component should be considered as unnecessary or ineffectual stimulant by such method of application as my experiments. Again, if I have to criticize the tuberculin treatment of the tuberculosis patients from my experiments. I should say that in an ordinarily used method, or the method in which the patients

are treated with as little tuberculin reaction as possible or no reaction, the injection of the substance contained in tuberculin which produces tuberculin reaction is not effective.

To sum up, by the tuberculin treatment (continuary vaccination), to use a small quatity of tuberculin by diluting it, is not effective. Rather, by using a large quantity of the second component in which the substance which produces the unnessary tuberculin reaction is removed from tuberculin, we might check the progress of tuberculosis or even cure it.

To mention some contributions of similar investigation.

1. According to the report of Armand delille, the ether extract of bacillus body accelerates the caseous degeneration, and the chroloform extracts works toward sclerosis.

2. Beck collect fat and lipoid from acid resisting bacillus and made the immunity experiment. The result was negative.

3. The therapeutic effect of the action of chaulmugra oil on the leprous node is demonstrably recognized. But it is not determined yet whether the action is immunoserological or not.

4. *Deycke*'s nastin is another instance. He found that the patients of leprosy was cured of the node when they had parasitic streptthrix. He collected neutral fat out of this thread like bacillus and found a curative action in this substance. He collected a similar substance from tubercle bacillus and applied it to leprous patient and found it had some effect on this disease.

5. *Much* proposed the partial immunity theory which he formulated by adding the immuno-biological consideration of his own to *Deycke*'s discovery. By using lactic acid, he separated water soluble substance, albumen, neutral fat, fatty-acid and lipoid from tubercle bacillus body. He supposed them to be partial antigen. Although there partial antigens have no immunability in themselves, there will appear immunability when proteinous fat and similar substances are conbined together. He insisted that he found this fact by immunity experiments of experimental tuberculosis.

6. *M. Kitagawa* reported that there is ulcer producing property in alcohol insoluble ether soluble component of the tubercle bacillus, body, and that alcohol soluble component has the action to cure ulcer. He called the former toxin and the latter anti-toxin component. It is very interesting exeriment. But since it is a sort of prediction and no detailed account is given, the author is not in a situation to criticize it.

7. Besides these, although we can not find any logical connection with this investigation, we must remember that there are many reports which suppose that the products of bacillus attack bacillus itself in a special manner. The following are the latest literatures concerning this investigation.

8. *Rosenbach* used tuberculin, which is digested by growth of tryphochyton hollosericum.

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Eperiment of III Chapter (This table is demonstrated here for the example of my experimental method)

	method of treatment		No. of guinea pigs	13/VII bodyweight	31/VIII bodyweight	13/VII quantity of inoculated T.b. bacilli		[illegible]	inguinal gl. right	inguinal gl. left	[illegible]	retrosternal gland	retro-[illegible] gland	portal gland	liver	lung		spleen	note
A	Subcutaneous injection with [illegible] tuberculin	before inoculation of T.b. bacilli every 1 cc 1/VII, 3/VII, 5/VII, 8/VII, 10/VII; after inoculation of T.b. bacilli every 0.5cc 13/VII, 15/VII, 17/VII, 19/VII, 21/VII, 24/VII, 26/VII, 29/VII, every 1cc 1/VIII, 8/VIII, 15/VIII, 25/VIII	700	250	250	1mg 1000	dissected after 50 days from inoculation of T.b. bacilli	small ulcer	beens cheesy-deg +	small [illegible] ch. deg ++	–	rice ch. deg –	a little hypertroph ±	–	a few nodes +	±	0.5 g	Macroscopically a few nodes [illegible] (±)	Degree of increase number of tuberculous nodes & degree of necrosis in the centrum of tuberculous nodes is following: D (controllgroup) > B > A > C
			312	300	350	,,		[illegible]	small beens ch. deg +	rice ch. deg ±	little hypertroph (+)	–	–	–	–	±	0.5 ,,	Smooth, macroscopically no T.b. node (–)	
			317	400	350	1 ,, 100		[illegible] + abscess	rice ch. deg –	2x rice ch. deg –	–	2x rice ch. deg –	hypertroph ch. deg ±	2x rice ch. deg –	very few nodes +	±	0.45 ,,	[illegible] in centrum of [illegible] (±)	
			200	500	400	,,		not visible	3x rice ch. deg –	small beens ch. deg –	–	–	–	small beens ch. deg –	±	±	0.7 ,,	a few milliar T.b. [illegible] nodes (++)	
			316	450	400	1 ,, 10		[illegible]	rice ch. deg –	2x rice ch. deg –	–	–	–	–	many nodes ++	±	0.7 ,,	Macroscopically a few T.b. nodes [illegible] (+)	
			108	250	310	,,			–	small beens ch. deg –	little hypertroph ch. deg –	–	–	pea ch. deg –	,, ++	±	0.75 ,,	Many milliar T.b. [illegible] many T.b. nodes (++)	
B	Subcutaneous injection with the sediment of tuberculin by alcohol, which is digested by pancreatin	the same with A	139	250	320	1 ,, 1000		abscess + [illegible]	small beens ch. deg ±	small beens ch. deg +	–	small beens ch. deg +	small beens ch. deg ±	–	a few nodes ,, +	±	0.7 ,,	Milliar [illegible] (++)	
			419	320	325	,,		,,	±	pea ch. deg ++	–	–	–	small [illegible] ch. deg (–)	–		0.5 ,,	Smooth, macroscopically [illegible] node (–)	
			315	450	450	1 ,, 100		,,	a little hypert ch. deg –	a little hypertroph ch. deg –	–	small beens ch. deg +	–	small [illegible] ch. deg ±	a few nodes necrotic ++		0.8 ,,	Milliar T.b. nodes few (+)	
			318	370	350	,,		[illegible] ulcer	rice ch. deg –	pea ch. deg ++	–	,, +	–	beens ch. deg ±	a few nodes +		0.7 ,,	,, (+)	
			414	320	260	1 ,, 10		abscess +	small beens ch. deg ±	2x rice ch. deg –	–	–	–	small beens ch. deg ±	very many nodes +++		0.8 ,,	Milliar T.b. [illegible] (++)	
			2	400	380	,,		,,	pea ch. deg ++	pea ch. deg ++	–	–	–	pea ch. deg ±	,, +++		0.8 ,,	Many milliar T.b. [illegible] centrum of nodes necrosis evident (+++)	
C	Subcutaneous injection with alcohol soluble substance of tuberculin which is digested by pancreatin	the same with A	417	280	420	1 ,, 1000		abscess + ulcer	½ rice ch. deg –	small beens ch. deg ±	–	–	–	–	±	±	0.8 ,,	T.b. nodes not evident (±)	
			317	300	320	,,		,,	–	–	–	–	hypertroph ch. deg –	–	many nodes ++	±	0.8 ,,	(±)	
			415	300	400	1 ,, 100		[illegible] ulcer	–	small beens ch. deg +	–	–	–	–	a few nodes +	±	0.7 ,,	(±)	
			311	330	/	,,		/	/	/	/	/	/	/	/	/	/	/	
			300	400	440	1 ,, 10		[illegible] ulcer	–	small beens ch. deg –	–	–	–	–	many nodes ++	±	0.75 ,,	A few milliar T.b. (+)	
			17	290	360	,,		abscess + ulcer	–	small beens ch. deg +	–	beens ch. deg ++	–	–	a few nodes +	±	0.5 ,,	T.b. node smooth, not evident (–)	
D	Controll		411	360	410	1 ,, 1000		small ulcer	pea ch. deg ++	pea ch. deg ++	a little hypertroph ch. deg –	beens ch. deg +	beens ch. deg +	small beens ch. deg ±	a few nodes ++	±	0.7 ,,	T.b. node not evident (±)	
			416	300	355	,,		abscess + ulcer	beens ch. deg ±	pea ch. deg ++	a little hypertroph ch. deg –	small beens ch. deg +	hypert. ch. deg –	pea ch. deg +	++	±	0.5 ,,	few milliar T.b. node (+)	
			412	250	350	1 ,, 100		small ulcer	,, ±	small beens ch. deg +	–	,, +	small beens ch. deg ++	,, ++	numerous milliar T.b. +++	±	0.7 ,,	,, (++)	
			313	320	350	,,		abscess + ulcer	small beens ch. deg +++	pea ch. deg +++	small beens ch. deg +	,, +	hypertroph ch. deg ±	,, ++	a few nodes +	few nodes +	0.75 ,,	Knotty tubercle, centrum of nodes necrotic (+++)	
			413	420	455	1 ,, 10		,,	pea ch. deg ++	½ broad beens ch. deg +++	ch. deg ±	–	–	,, ±	some nodes ++	±	0.8 ,,	Many small nodes (++)	
			418	360	310	,,		,,	3x rice ch. deg ++	beens ch. deg +	hypertroph ch. deg –	small beens ch. deg ++	–	½ broad beens ch. deg ++	numerous nodes necrotic +++	±	0.7 ,,	Many milliar T.b. centrum of nodes necrotic +++	

Chemistry

Über die glykolytische Kraft des Blutes.

Von

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Über den glykolytischen Vorgang im Blute gehen die experimentellen Ergebnisse von vielen Autoren so weit auseinander, dass man noch nicht zu einem bestimmten Schluss kommt. Besonders darüber herrschen zwei ganz entgegengesetzte Ansichten, ob die hämolysierten Blutkörperchen noch glykolytische Kraft beibehalten oder nicht. So habe ich die Untersuchungen über solche noch nicht ganz aufgeklärte und widerspruchsvolle Glykolyse des Blutes angestellt.

Als Versuchstier wurde hauptsächlich das Kaninchen verwendet. Das aus der Ohrvene entnommene Blut wurde in einen Kolben mit Glasperlen gebracht und defibriniert. Mittels der Zentrifuge wurde das Blutserum abgetrennt und die mit der physiologischen Kochsalzlösung 3–4 mal ausgewaschenen Blutkörperchen wurden in derselben Kochsalzlösung aufgeschwemmt. Weiter wurde diese Aufschwemmung der Blutkörperchen mit der 10% Traubenzucker enthaltenden isotonischen Kochsalzlösung von einer gewissen Menge versetzt, welche dann in einen 37°C Brutschrank zur Beobachtung der Zuckerverlust gestellt wurde. Zur Feststellung des Zuckergehalts wurde das Mikroverfahren von Bang befolgt. Die Blutkörperchenzahl, welche bisher fast ausser Betracht gelassen wurde, wurde in meiner Beachtung genommen, vor und nach jeder Untersuchung mit Hämozytometer berechnet, um mit der Menge des verminderten Zuckers kollationiert zu werden. Die Bakterienfreiheit der streng aseptisch behanderten Proben wurde stets durch aerobene und anaerobene Kultur sichergestellt. Die Ergebnisse der Experimente sind kurz zusammenzufassen wie folgt.

1. Blutserum und Plasma haben keine glykolytische Kraft.

2. Die glykolytische Kraft der Erythrozyten des gesunden Kaninchens hängt wenig von der Zuckerkonzentration, beim Gehalt von 0.155–0.317%, aber hauptsächlich von der Zahl der Erythrozyten ab. Die Erythrozytenzahl und die gespaltene Zuckermenge sind in parallelem Verhältnis, namentlich wenn es sich um das gleiche Blut handelt.

3. Die glykolytische Kraft der Blutkörperchen ist von deren Medium beeinflusst worden. Im Serum oder Gemisch des Natriumphosphats von Blutalkaleszenz ist sie stärker als in der physiologischen Kochsalzlösung oder Ringerschen Lösung, d. h. etwa 2 mal so gross wie in den beiden letzteren.

4. Bei der Hämolyse nach der verschiedenen chemischen und physikalischen oder immunologischen Methode nimmt die glykolytische Kraft je nach dem Grade der Hämolyse ab. Bei der vollkommenen Hämolyse verschwindet sie auch vollständig, und im Gegensatz zum Rona u. Arnheim's Experimente tritt sie nicht wieder in Erscheinung durch den gleichzeitigen Zusatz des Natriumphosphatgemisch von Blutalkaleszenz.

5. Zyan und Toluol üben keinen Einfluss auf die Glykolyse aus, solange deren Konzentration nicht ausreicht, um eine Hämolyse herbeizuführen. Alkohol, Äther, Chloroform, Chininhydrochlorid und die konzentrierte Kochsalzlösung verhalten sich wie diese beiden. Die vorher mit absolutem Alkohol behanderten Blutkörperchen zeigen keine Glykolyse.

6. Der Abschluss des Sauerstoffs verändert die glykolytische Kraft der Blutkörperchen nicht. Sauerstoffzufuhr in grösserer Quantität ist auch von keinem Einfluss. Von gleichem Verhalten ist CO, Kohlenmonoxyd. Kohlensäure in grosser Menge stört die glykolytische Kraft, die sich aber bei der Sauerstoffzufuhr erholt.

7. Es hat den Anschein, dass die Glykolyse im Blute hauptsächlich von den Erythrozyten verursacht wird, da die Leukozyten, die in geringer Anzahl den Erythrozyten beigemengt sind, keine erkennbare glykolytische Wirkung, wenigstes nicht in absolutem Mass, verrichten.

8. Die Erythrozyten in der Eiskammer behalten die glykolytische Kraft über 10 Tage lang bei, dagegen im Brutschrank von 37°C verlieren sie meistens nach 24 Stunden diese Kraft, wenn sie auch in der physiologischen Kochsalzlösung ohne Zuckerzusatz aufgeschwemmt worden sind oder durch die verschiedenen techinischen Manipulationen während des Versuchs in die günstigeren Zustände für ihre Lebensfunktion gestellt werden. Da ich noch durch eine biologische Untersuchung bestätigen konnte, dass eine innige Beziehung zwischen der glykolytischen Kraft zu der Lebensfunktion der Erythrozyten besteht, konnte das Bestehen der Glykolyse auch als Beweis für die Beurteilung des Lebens der Erythrozyten dienen.

Die biologische Untersuchung wurde wie folgt ausgeführt: Die ca. 100 ccm. frisch bereitete oder einen Tag in Eiskammer aufbewahrte, die glykolytische Kraft noch genug vorbehaltende Blutkörperchenaufschwemmung des Kaninchens wurde in die Bauchhöhre eines gesunden Kaninchens mit dem Erfolg injiziert, dass eine Polyzythämie vorübergehend in Erscheinung trat und keine Hämoglobinurie entleert wurde. Wenn die 24 Stunden lang im Brutschrank belassene, schon glykolytische Kraft verloren habende Blutkörperchenaufschwemmung von gleicher Menge zur Injektion gebraucht wird, so zerfallen die Blutzellen im injizierten Kaninchenskörper und im Gegensatz zum vorigen Fall kommt keine Polyzythämie, sondern deutliche Hämoglobinurie in Auftritt. (*Autref.*)

Über die glykolytische Wirkung des Harns.

Von

Dr. **Yoshikane Kawashima.**

(Aus der biochemischen Abteilung des Instituts
Vorstand: Dr. Teisuke Kohmoto.)

Wie bekannt ist, legen wir einen grossen Wert an der quantitativen Zuckerbestimmung im Harne ausser dem Blute bei der richtigen und zweckmässigen Behandlung von Diabetes mellitus. Da aber es klinisch nicht immer möglich ist, jedesmal den frischen Harn zu prüfen, so bestimmen wir meistens den Zuckergehalt in dem 24 Stunden lang gesammerten Tagesharn. Doch erfahren wir, dass dabei eine unvorsichtige Aufbewahrung von dem Harne, besonders bei hoher Temperatur, eine merkliche Zerstörung des Zuckers darin verursacht. Wenn das der Fall ist, so fällt leicht eine Täuschung auf die Behandlung der Krankheit. Hierbei ist es eine offene Frage zu entscheiden, ob diese Zuckersplatung von den Wirkungen der zufällig gemischten Mikroorganismen oder eines eigenartigen glykolytischen Ferments herrührt, dessen Vorhandensein im Harne schon von G. Pierallini negiert wurde. Über solche Frage einen bestimmten Schluss zu erlangen, scheint es mir nicht überflüssig, die Experimente in diesem Gebiete auszuführen. Dafür wurde die folgenden Untersuchungen angestellt.

Versuchsmethode: Zu verschiedenen Zuckerharnen, deren Reaktion zuweilen mit dem Natriumkarbonat vorher alkalisch gemacht wurde, wurde das Toluol hinzugefügt, bis eine dünne Toluolschicht die ganze Oberfläche des Harns überdeckte. Diese Harne in Flaschen wurden in Zimmertemperatur, Eiskammer oder Brutofen zeitlang stehen gelassen, damit ihrer Zuckergehalt serienweise bestimmt wurde. Als die Kontrollproben kommen die Harne, welche ohne den Toluolzusatz aufbewahrt wurden, zur Anwendung. Zur Zuckerbestimmung steht die Pavy-Kumagawa-Sudosche Methode hauptsächlich in Verdienst. Jedesmal wurde das Filtrat des aufbewahrten Harns zur Zuckerbestimmung verwendet.

Die Temperatur in allen folgenden Tabellen zeigt die des Zimmers, in dem die Harnflaschen gestanden sind. Die Temperatur in der Eiskammer schwankt zwischen 4°C und 10°C mehr.

Versuchsergebnisse.

I. Normaler Harn, mit dem Traubenzucker und Toluol versetzt.

Table I.

Dat.	Zimmertemperatur			Eiskammer		
	Temperatur (C)		Reaktion des Harns	Zuckergehalt in g/dl.	Reaktion des Harns	Zuckergehalt in g/dl.
	Maximum	Minimum				
26/VI	23.5	21.5	schwach sauer	3.30	schwach sauer	3.31
28/ „	24.5	21.5	„	3.31	„	3.31
3/VII	29.5	25.0	„	3.31		
8/ „	29.5	27.0	„	3.31	„	3.31
13/ „	28.5	26.5	„	3.31	„	3.31
19/ „	34.5	28.0	„	3.31	„	3.31
24/ „	28.5	26.0	„	3.31	„	3.31

Wie aus der Tabelle I ersichtlich ist, zeigte der Harn einen Monat hindurch ganz gleichen Zuckerwert an.

II. Normaler Harn 150 ccm + Toluol 10 ccm + Traubenzucker.
(in 37°C Brutschrank gestellt.)

Tabelle II.

Dat.	Zuckergehalt in g/dl.	
	A	B
24/VIII	1.30	1.33
25/ „	1.30	1.33
27/ „	1.30	1.33

A = schwach sauer
B = mit 0.1 gr. Natriumkarbonat versetzt und alkalisch gemacht.

Wie die Tabelle II. zeigt, bleiben die allen Proben voll 3 Tage lang in der ganz gleichen Zuckermenge.

III. Gonorrhoischer Zystitisharn mit dem Zusatz von Traubenzucker und Toluol.

Aus der Tabelle III. kann man ersehen, dass der Zucker in zystitischen Harn (Gonorrhöe) auch nach einem Monate keine Veränderung erfahren hat.

Tabelle III.

Dat.	Zimmertemperatur (C)		Reaktion des Harns	Zuckergehalt in g/dl.
	Maximum	Minimum		
14/VII	29.5	27.0	[illegible]	3.74
15/ „	33.5	26.0	„	3.74

16/VII	30.5	28.0	sauer	3.74
19/ „	34.5	28.0	„	3.74
24/ „	28.5	26.0	„	3.74
29/ „	28.5	27.0	„	3.74
9/VIII	27.5	26.5	„	3.75
17/ „	28.5	26.5	„	3.75

IV. Versuch mit dem zystitischen Harn von Kolibazillen, zu dem Traubenzucker und Toluol hinzugesetzt wurden.

Tabelle IV.

Dat.	Zimmertemperatur (C)		Reaktion der Harns	Zuckergehalt in g/dl.
	Maximum	Minimum		
14/VIII	28.5	27.5	sauer	3.48
15/ „	28.5	27.0	schwach alkalisch	3.48
16/ „	28.5	27.0	alkalisch	3.48
17/ „	28.5	26.5	„	3.48
19/ „	27.0	26.0	stark alkalisch	3.45
21/ „	28.0	26.5	„	3.45
28/ „	26.0	25.0	„	3.39
6/IX	28.5	26.5	„	2.33

Wie aus der Tabelle IV. ersichtlich ist. war der Zuckergehalt des betreffenden Harns mehrere Tage lang ganz stationär. Im nachherigen Verlaufe war er aber etwas vermindert, was vielleicht dabei durch die Umwanderung in die starke Alkalität des Harns hervorgerufen wurde. Daraus kann man sagen, dass der Zucker in Bakteriurie nicht so leicht in Spaltung kommt, wenn man die Bakterienwirkung durch den Toluolzusatz im voraus verhütet.

V. Versuch mit dem Zuckerharn des pankreasexstirpierten Hundes, mit Toluol versetzt.

Tabelle V.

Dat.	Zimmertemperatur in der Untersuchungszeit (C)	A		B	
		Reaktion des Harns	Zuckergehalt in g/dl.	Reaktion des Harns	Zuckergehalt in g/dl.
12/VI	23.0	sauer	3.50		
14/ „	23.0	„	3.50	schwach sauer	5.13
15/ „	26.0			„	5.13

16/VI	25.0			schwach sauer	5.13
17/„	28.5	sauer	3.50		
21/„	25.0			sehr schwach sauer	5.13
26/„	23.5			schwach alkalisch	5.13
29/„	24.5	„	3.51		
3/VII	29.5			„	5.14
8/ „	29.5			„	5.13
10/ „	28.5	„	3.51		
13/ „	28.5			„	5.13

A=Azeton und Azetessigsäure sind negativ.
B=Diese beiden sind positiv.

Wie auf der Tabelle V. veranschaulicht ist, über den Harn des pankreasexstirpierten Hundes wurde sein Zuckergehalt 31 Tage lang untersucht, keine Verminderung zu zeigen.

VI. Der mit Toluol versetzte Harn von den Diabetikern.

Fall 1.

A=in Zimmertemperatur gestanden.
B=in Eiskammer gestellt.
C=mit der Essigsäure angesäuert und in Eiskammer gelegt.

Tabelle VI. (Fall 1.)

Dat.	A				B		C	
	Zimmertemperatur (C)		Reaktion des Harns	Zuckergehalt in g/dl.	Reaktion des Harns	Zuckergehalt in g/dl.	Reaktion des Harns	Zuckergehalt in g/dl.
	Max.	Min.						
23/VI	23.0		alkalisch	3.84	alkalisch	3.84	sauer	3.80
24/„	23.0	22.0	„	3.84	„	3.84	„	3.80
26/„	23.5	21.5	„	3.84	„	3.84		
28/„	24.5	21.5	„	3.84			„	3.80
2/VII	27.5	23.5	„	3.84	„	3.84	„	3.86
7/„	35.5	26.5	„	3.84	„	3.80	„	3.86
12/„	28.5		„	3.84	„	3.80	„	3.82
17/„	33.0	27.5	„	3.84	„	3.80	„	3.82
22/„	29.5	27.5	„	3.84	„	3.80	„	3.82

Fall 2.

Bei diesem Falle ist der Zuckergehalt durch die Behandlung allmählich sich vermindert und der geringer Menge Zucker enthaltende Harn wurde zur Probe angestellt, wie die folgende Tabelle zeigt.

Tabelle VII. (Fall 2.)

Dat.	Zimmertemperatur (C)		Reaktion des Harns	Zuckergehalt in g/dl.
	Maximum	Minimum		
23/VI	23.0		sauer	0.73
24/ „	23.0	22.0	„	0·73
26/ „	23.5	21.5	„	0.72.
28/ „	24.5	21.5	„	0.71
2/VII	27.5	23.5	schwach sauer	0.71
7/ „	25.5	26.5	„	0.71
12/ „	28.5		„	0.72
17/ „	23.0	27.5	„	0.71
22/ „	29.5	27.5	„	0.72
24/ „	28.5	26.0	„	0.71

Tabelle VIII. (Fall 3.)

Dat.	Zimmertemperatur (C)		Reaktion des Harns	Zuckergehalt in g/dl.
	Maximum	Minimum		
15/VII	33.5	26.0	schwach sauer	2.44
16/ „	30.5	28.0	„	2.44
17/ „	33.0	27.5	„	2.44
22/ „	29.5	27.5	„	2.44
9/VIII	27.5	26.5	„	2.45
17/ „	28.5	26.5	„	2.45

Die Ergebnisse dieser drei Versuche (Tabelle VI-VIII) sind fast gleich den Ergebnissen der vorangehenden.

Die oben erwähnten Ergebnisse führen nun dazu, dass der Traubenzucker im Harne ziemlich lange Zeit nicht gespalten wird, wenn es sich um den durch Toluolzusatz vor der Fäulnis verhütet aufbewahrten Zuckerharn handelt, obgleich er mit Bakterien, z. B. Kolibazillus, vermischt, oder seine Reaktion sauer oder alkalisch ist, vorausgesetzt von leichter Alkalität, die den Zucker in Spaltung nicht kommen lässt.

Nun wurden die Untersuchungen über den ohne Toluolzusatz aufbewahrten Zuckerharn ausgeführt.

VII. A. Der normale Harn mit dem Zuckerzusatz. (Harnflasche dicht geschlossen.)

Tabelle IX.

Dat.	Zimmertemperatur					Eiskammer		
	Temperatur(C)		Reaktion des Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %	Reaktion des Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %
	Max.	Min.						
26/VII	33.0	27.0	schwach sauer	3.43	0	schwach sauer	3.43	0
27/ „	30.0	27.5	„	3.39	1.2	sehr schwach sauer	3.42	0.3
28/ „	29.0	27.0	mittelmässig sauer	3.20	6.7	neutral	3.42	0.3
29/ „	28.5	27.0	sehr schwach sauer	2.90	15.5	sehr schwach sauer	3.22	6.1
30/ „	28.0	27.0	schwach alkali	1.95	43.1	„	2.67	22.2
31/ „	28.5	27.5	schwach sauer	1.00	70.8	schwach sauer	2.13	37.9
1/VIII	29.5	28.0	mittelmässig sauer	0.50	85.4	mittelmässig sauer	1.56	54.5
3/ „	29.0	28.0	„	0.32	90.7	„	0.66	80.8
5/ „	27.5	26.0				„	0.34	90.1

In Zimmertemperatur gestellter Harn: Seit dem 2. August war die Menge des zu untersuchenden Harns so gering, dass die quantitative Bestimmung ungenügend ausführbar war. Am 5. war die Trommersche Reaktion schwach positiv. Am 7. fiel sie negativ aus.

B. Der normale Harn mit dem Zuckerzusatz. (Die Flaschenöffnung ist offen gestanden.)

Tabelle X.

Dat.	Zimmertemperatur					Eiskammer		
	Temperatur(C)		Reaktion des Harns	Zuckergehalt in g/dl	Zuckerabnahme in %	Reaktion des Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %
	Max.	Min.						
2/VIII	28.5	27.5	sauer	3.17	0	sauer	3.17	0
3/ „	29.0	28.0	„	2.13	1.3	„	3.17	0
4/ „	28.5	27.0	schwach sauer	2.72	14.2	schwach sauer	3.20	(+)0.9
5/ „	27.5	26.0	alkali.	1.72	45.7	„	3.13	1.3
6/ „	28.0	26.5	„	0.53	83.3	„	2.99	5.7

7/VIII	27.0	26.0	stark alkali.	0.34	89.3	mittelmässig sauer	2.87	9.5
8/ „	27.0	26.0	„	0.31	90.2	„	2.86	9.8
11/ „						„	2.86	9.8
13/ „						„	2.26	28.7
16/ „						„	1.82	42.6
18/ „						„	1.49	53.0
20/ „						„	1.05	66.9

Beim Harn in Zimmertemperatur war die Nylandersche Reaktion am 11. August spur positiv.

C. Ein Teil des Harns B wurde mit dem Natriumkarbonat versetzt, und dann zur Probe angestellt. (1 gr. Na_2CO_3 zu 150 ccm Harn.)

Tabelle XI.

Dat.	Zimmertemperatur					Eiskammer		
	Temperatur(C)		Reaktion des Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %	Reaktion des Harns	Zuchergehalt in g/dl.	Zuckerabnahme in %
	Max.	Min.						
2/VIII.	28.5	27.5	stark alkali.	3.17	0	stark alkali.	3.17	0
3/ „	29.0	28.0	„	3.10	2.2	„	3.15	0.6
4/ „	28.5	27.0	„	2.86	9.8	„	3.10	2.2
5/ „	27.5	26.0	„	2.48	21.8	„	3.03	4.4
6/ „	28.0	26.5	„	2.20	30.6	„	2.92	7.9
7/ „	27.0	26.0	„	1.79	43.5	„	2.84	10.4
8/ „	27.0	36.0	„	1.39	56.2	„	2.67	15.8
10/ „	27.5	26.5	„	0.58	81.7	„	2.52	20.5
11/ „	27.5	26.5	„	0.50	84.2			
12/ „	28.0	27.0	„	0.39	87.7			
13/ „						„	2.22	30.0
16/ „						„	1.92	39.4

Harn in Zimmertemperatur: Am 11. August war die Nylandersche Reaktion schwach positiv, am 12. fast negativ.

Bei diesen drei Versuchen ohne Zusatz von Toluol wurde der Traubenzucker allmählich zerstört, und der abgenommene Zucker zeigte an Menge keinen Unterschied zwischen der Verschliessung und der Eröffnung des Mundes der Harnflasche. In der Zimmertemperatur verminderte sich der Zucker nach 24 Stunden 1.2 2.2%, nach mehreren Tagen aber ging ein grösster Teil des hinzugefügten Zuckers schon zerfallen. Bei dem Harn in Eiskammer war die Zuckerzerstörung so schwächer als die beim vorigen, dass der Zuckergehalt anfangs 1–2 Tage lang konstant war

und danach allmählich abnahm. Bei dem mit Natriumkarbonat stark alkalisch gemachten Harn war die Zuckerspaltung anfangs etwas stärker, später aber schwächer als die anderen, was aus der Tabelle ersichtlich ist. Ob dieser Vorgang vielleicht dadurch bedingt ist, dass die starke Alkalität für die Entwicklung der im Harn hineingemischten Bakterien ungünstig wirkt, bleibt jedoch hier wegen des nur einen Versuchsfalls und der Entbehrung der vergleichenden Bakterienkultur nicht sicher entschieden.

VIII. Versuch mit dem Harn eines Diabetikern ohne Toluolzusatz. (in der Zimmertemperatur stehen gelassen.)

A = sauer. B = mit dem Natriumkarbonat schwach alkalisch gemacht.

Tabelle XII.

Dat.	A					B		
	Temperatur(C)		Reaktion das Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %	Reaktion des Harns	Zuckergehalt in g/dl.	Zuckerabnahme in %
	Max.	Min.						
6/VIII	28.0	26.5	sauer	5.63	0	schwach alkali.	5.63	0
7/ „	27.0	26.0	„	5.63	0	„	5.59	0.7
8/ „	27.0	26.0	„	5.00	11.2	„	4.74	15.8
9/ „	27.5	26.5	„	4.65	17.4	alkali.	3.77	33.0
10/ „	27.5	26.5	„	4.08	27.5	stark alkali.	2.56	54.5
11/ „	27.0	26.5	„	3.96	29.7	„	1.72	69.4
12/ „	28.0	27.0	„	3.54	37.1	„	0.86	84.7
13/ „	28.0	27.0	„	3.33	40.9	„	0.20	96.4
15/ „	28.5	27.0	schwach sauer	2.50	55.6			
17/ „	28.5	26.5	stark alkali.	0.55	90.2			

Wie die Tabelle XII. zeigt, ist das Ergebnis des Versuchs fast gleich wie die vorhergehenden. Bei der sauren Reaktion des Harns bleibt der Zuckergehalt während ersten einen Tages unverändert. Bei der alkalischen lässt er sich am zweiten Tage, d. h. nach 24 Stunden, 0.7% vermindern und kommt 6 Tage danach fast zur Null, in Abnahme von 96,4%, während die Zuckerabnahme beim sauren Harn dabei 40.9% beträgt.

Zusammenfassung.

1. Der Traubenzucker im Harne kommt nicht in der Zerstörung etwa einen Monat hindurch, wenn man ihn durch den Zusatz von Toluol vor Fäulnis verhütend aufbewahrt, was gilt auch bei den Bakteriurien mit Zuckerzusatz ganz gleiches.

2. Dagegen aber spaltet der Zucker sich allmählich, wenn der Harn ohne Toluolzusatz spontan gelassen wird. Bei dem Harn in Zimmertemperatur (33° 27°C) vermindert die Zuckermenge nach einem Tage 1.2–2.2%, beim Harn in Eiskammer bleibt sie während 1–2 Tage fast unverändert, und dann tritt die Spaltung bei den beiden Harnen mehr rasch in Erscheinung. Natürlich ist sie schwächer bei dem letzteren als bei dem vorigen. Dabei scheint eine schwache Alkalität des Harns auf die Zuckerspaltung etwas beschleunigend, eine starke dagegen etwas verzögernd zu beeinflussen.

3. Daraus kann man schliessen, dass der Harn kein glykolytisches Ferment enthält, und die Bakterien bei der Zuckerzerstörung im Harne die hauptsächlichste Rolle spielt.

Zum Schluss erlaube ich mir, Herrn Dr. T. Kohmoto für seine Leitung bei dieser Arbeit, Herrn Dr. Z. Hatta, Herrn Dr. K. Sakaguchi und Herrn Dr. H. Negishi, die mir den Harn des pankreasexstirpierten Hundes oder Diabetikern und die Bakteriurien zum Untersuchungsmaterial gaben, für ihre Güte meinen ergebensten Dank auszusprechen.

Über die Wirkungen der Spaltungsprodukte des polierten Reises auf den Froscherzen, besonders mit der Berücksichtigung von Vitamin (B).

Von

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(Veröffentlicht am 28. Oktober 1922.)

Nunmehr kann man zweifellos behaupten, dass der Mangel an Vitamin B auf der Ursache der Beriberi, Polineuritis gallinarum, eine wichtigste Rolle spiele. Nach der pathologisch-anatomischen Veränderungen der Kakkeleiche und derselben klinischen Befunden bzw. therapeutischen Erfahrungen ist es zu viel schwer, die ganze Processe nur mit dem Vitaminmangel selbst zu erklären. Dann drängt sich uns von selbst die Vorstellung auf, dass das Aufnahme des polierten Reises etwaige abnormen chemischen Vorgäng im Darmtraktus erregen, und die Resorption derselben Spaltungsprodukte den Polineuritis verursache, wenigstens die Erkrankung befördere, und dabei das Vitamin den schädlichen Verdauungsprodukten entgegen wirke.

Schon früher bestätigte Ich mit meinem Mitarbeiter T. Kohmoto eine sehr interessante Tatsache, dass die mit den Spaltungsprodukten zwangsmässig gefütterten Hühner doppelt so früh sich erkrankten als die nur mit dem polierten Reis ernährten. (die Kontrolle). Hierbei schafften wir die Spaltungsprodukte des polierten Reises, den natürlichen Vorgängen nachahmend wie folgendes: der mit dem Wasser wiederholt gewaschene kleienfreie Reismehl einmal gekocht, dann ihm Diastase, Pankreatin und der Kot von Kakkekranken hinzugefügt und im Brutofen etwa eine Woche lang stehen gelassen. Daraus schien es uns sehr wahrscheinlich, dass die Spaltungsprodukte des polierten Reises an inniger Beziehung mit dem Polineuritis gallinarum stehe. Wenn doch das der Fall wäre, soll das Vitamin auch irgendeine hemmende Beeinflussung auf die schädigende Wirkung der Spaltungsprodukte haben. Angesichts dieser Vorstellungen erwünscht es uns noch weiter zu untersuchen.

Jetzt durch Eindampfen des Dialysates von den wie oben zubereiteten Spaltungsprodukten* auf dem Wasserbad bekommt man eine sehr stark saure, dick sirpöse Flüssigkeit, die bei der Versuche im Darmlumen von den Versuchtieren

oder subcutan die Injektion ausgeführt wurde. Als Versuchstiere steht das Frosch am bequemlichsten uns im Verdienst. Die weiteren Versuche wurde einerseit mit der Kontrolle von einigen organischen Säuren, Essig- Milch- und Buttersäure, die ebensogleich starke Acidität wie die des eingedampften Dialysats haben, andererseits dieselbe mit recht genauer Neutralisierung des Dialysats mit Lauge angestellt. Hierbei wurde das Vitamin B (nach Tsukiye oder Suzuki's Orisanin) mit dieser sauren Flussigkeit gleichzeitig oder nacheinander an den Tieren verabreicht. Aber als man zur Zeit das Vitamin noch nicht rein krystallinisch isolieren kann, tut es uns bedürftig zu entscheiden, ob die Wirkung von Vitamin dem eigentlichen Bestandteile desselben oder dem zufällig gemischten zu zuschreiben sei. Um den Einwand doch zu vermeiden, wiederholt ich die Versuche mit der Orizaninlösung, die, wie schon jemand erkannt, durch Erhitzung eine Stunde lang im Autoclaven bei 140°C oder durch Veraschung die eigentliche Wirkung vernichtet wurde. Die Resultate sind wie folgendes:

1) Die Spaltungsprodukte von dem polierten Reises wirken sicher gegen das Froschherzen giftig, und die Herzschläge werden allmählich verlangsamt, um schliesslich zum grösstenteil diastolisch still zu stehen.

2) Das Vitamin wirkt sehr erfolgreich auf das Froschherzen, der schädlichen Wirkung der Spaltungsprodukte entgegend. Dabei steigert die Zahl der nicht stillstehenden Fällen von Froschherzen am günstigsten um 5–9 Fach, durchschnittlich 3 fach so viel als die der Kontrolle, welche nur die Spaltungsprodukte gegeben werden.

3) Um das Vitamin den Spaltungsprodukten entgegen erfolgreich zu wirken, ist es notwendig, die beide Dosierung parallel gehen zu lassen.

4) Um sich die Gegenwirkung des Orizanins gut entwickeln zu lassen, ist es nötig, dies den Spaltungsprodukten voraus zu geben. Und zwar liegt das günstigste Zeitverhaltniss in 30 Minuten bis 2 Stunden.

5) Essig-, Milch- und Buttersäure wirken ebenso schädlich für das Froschherzen. Und das Orizanin zeigt auch hier eine Gegenwirkung, sei es bedeutend reducierten Grades, zu den säuren. Die Zahl von nicht stillstehenden Fällen der Froschherzen überwiegt die der Kontrolle um ca. 40–50%.

6) Mit den recht neutralisierten Spaltungsprodukten werden die Herzschläge nur verlangsamt; aber doch stehen sie nicht still. Wodurch ist es kaum möglich, es festzustellen, wie sich das Orizanin für diese Verhalten.

7) Weder das über 140°C gekochte noch das veraschte Orizanin zeigt sich nennenswerte schützenden Wirkungen gegen die von den Spaltungsprodukten. Das beweisst es, dass die Wirkung des Orizanins dem thermolabilen Bestandteile desselben recht zuschreiben soll.

(*Autoref.*)

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